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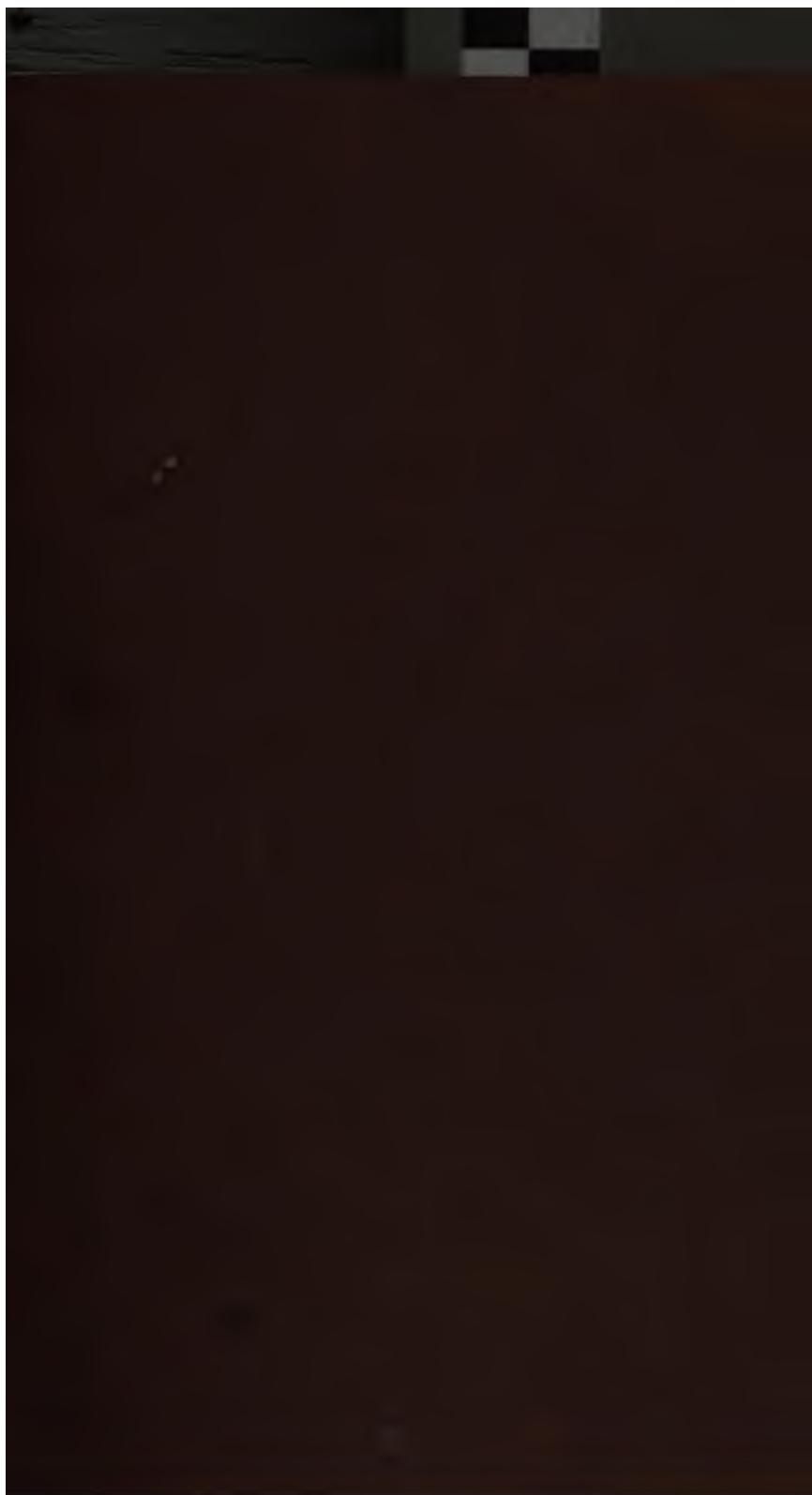


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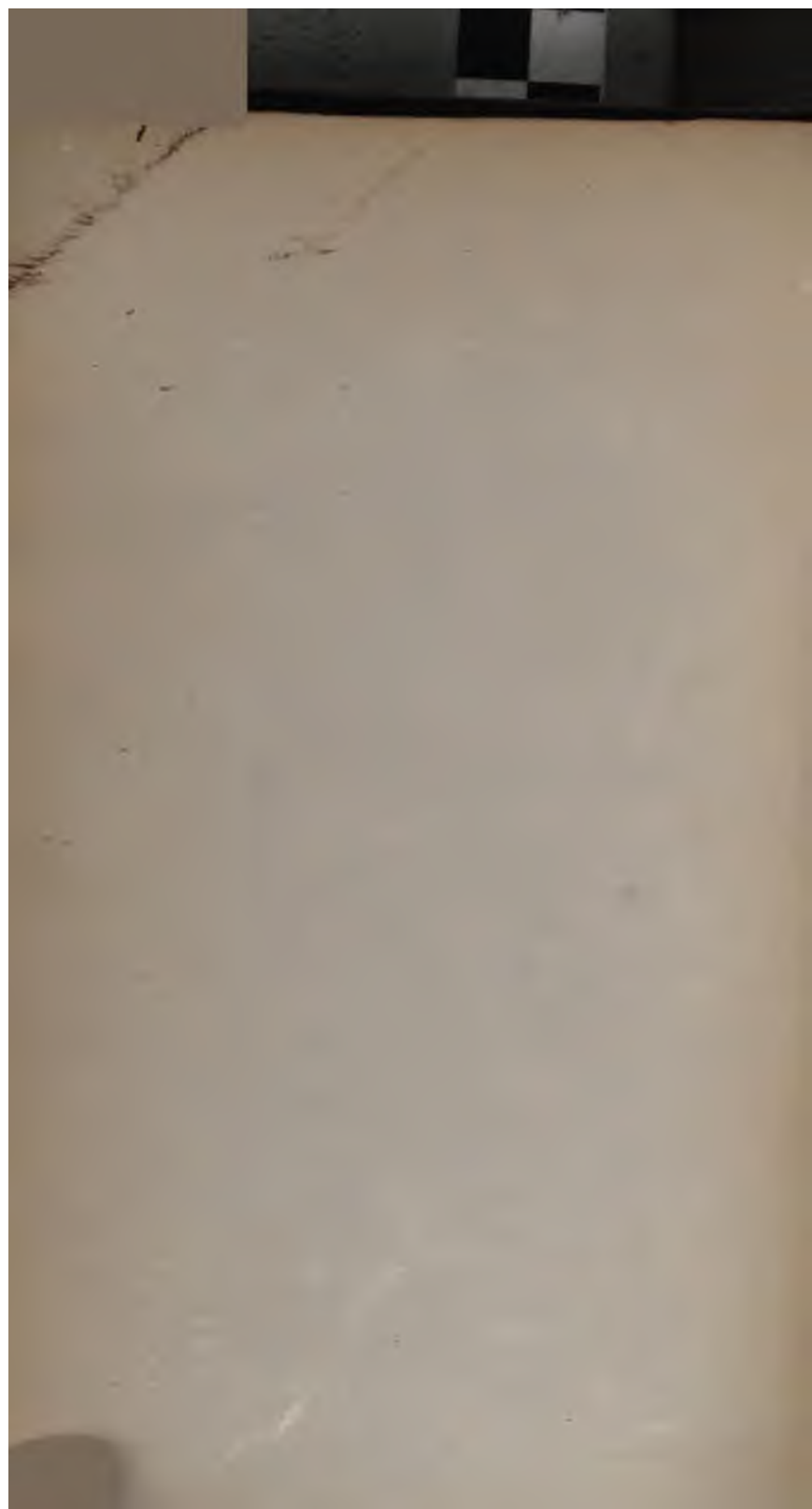
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*Stakley Vanderpool*













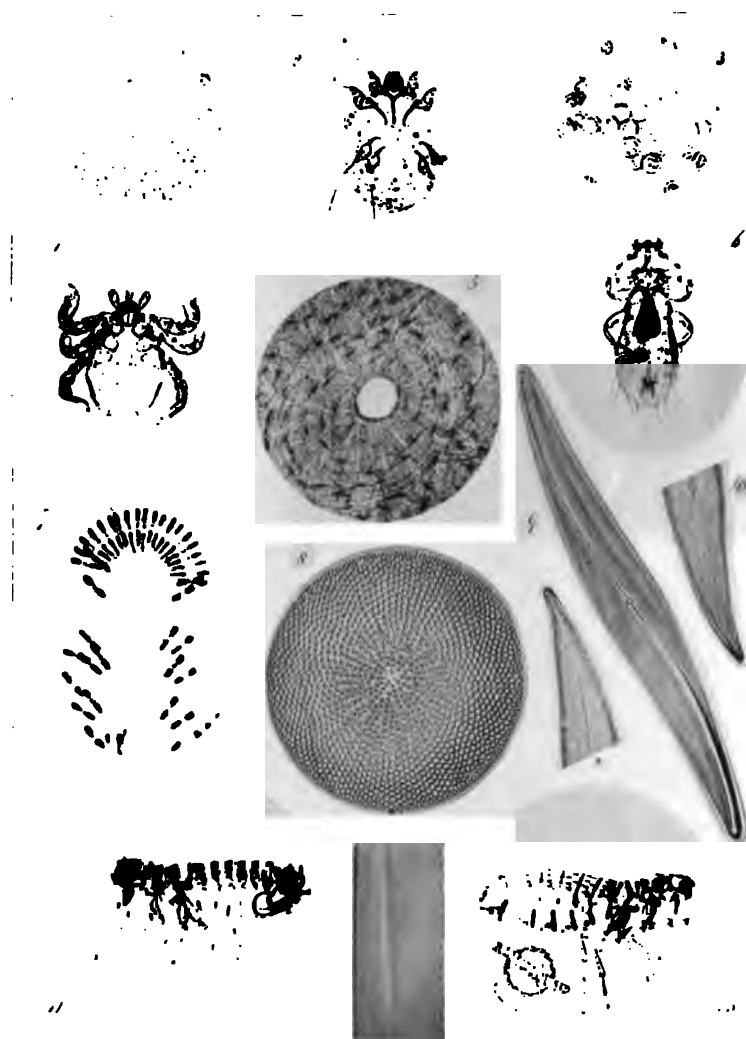


PLATE 10.

# EXPLANATION OF THE PLATE.

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|---|--------------------|
| 1. Pith of Stem of <i>Hyperandra</i> , transverse section ...       | 3/4 in. object ve. |
| 2. <i>Sarcopetalum</i> (young), transverse section ...              | 1/4 in. "          |
| 3. <i>Hyperandra</i> (young), transverse section, rapidly dried ... | 1/4 in. "          |
| 4. <i>Hyperandra</i> (young), transverse section ...                | 1/4 in. "          |
| 5. <i>Hyperandra</i> (young), transverse section ...                | 1/4 in. "          |
| 6. <i>Hyperandra</i> (young), transverse section ...                | 1/4 in. "          |
| 7. <i>Hyperandra</i> (young), transverse section ...                | 1/4 in. "          |
| 8. <i>Hyperandra</i> (young), transverse section ...                | 1/4 in. "          |
| 9. <i>Hyperandra</i> (young), transverse section ...                | 1/4 in. "          |
| 10. <i>Hyperandra</i> (young), transverse section ...               | 1/4 in. "          |



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# HOW TO WORK

WITH

## THE MICROSCOPE.

BY

LIONEL S. BEALE, M.B., F.R.S.,

FELLOW OF THE ROYAL COLLEGE OF PHYSICIANS, PHYSICIAN TO KING'S COLLEGE HOSPITAL,  
AND PROFESSOR OF PHYSIOLOGY IN KING'S COLLEGE, LONDON; HONORARY  
FELLOW OF KING'S COLLEGE.

*THIRD EDITION.*

Illustrated with Fifty-six Plates, containing upwards of 250 Figures  
and a Photographic Plate.



PHILADELPHIA :  
LINDSAY AND BLAKISTON.

1865.

The Author reserves the right of translating this Work

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## P R E F A C E .

It is now seven years since the first edition of this work was published, and during this short period very great advance has been made in many branches of microscopical inquiry, both in this country and on the Continent.

Since the publication of the original work, Messrs. Powell and Lealand have succeeded in making, at the request of the author, an object-glass magnifying 1,800 diameters. He hopes shortly to receive from them a power as much superior to this as the  $\frac{1}{16}$ th is to the old  $\frac{1}{16}$ th. Microscopical science is greatly indebted to these makers for the advances made by them upon several occasions in the manufacture of object-glasses, and in the construction of microscopes.

The author has considered it better to divide the work into *chapters* instead of *lectures*, but the original style has been retained, because it was thought to be well adapted for the description of practical details, in which clearness is of far greater importance than elegance of expression.

The book now contains more than twice the amount of work in the last edition. Many of the paragraphs have been re-written, and three new chapters, containing nearly one hundred pages, have been introduced. The number of plates has been increased from 32 to 56.


The author has still further improved upon the mode of injection and preparation of tissues advocated by him and now adopted by many observers. In this edition the details of the particular method of preparation carried out by the author in his investigations with the aid of the highest magnifying powers yet made, are for the first time published.

For the beautiful photograph which forms the frontispiece the author is indebted to his friend Dr. Maddox, who has also afforded him very great assistance in writing the chapter on

photography. This is one of the most valuable chapters in the book. It contains the results of many years' most earnest work, by one of the most successful workers in this department of photography. The detail of some of the photographic illustrations is so very minute, that many points cannot be seen by the unaided eye. A lens of low magnifying power has therefore been appended to the volume, to enable the reader to see the beautiful microscopical details which have been obtained by this mode of illustration, in which Dr. Maddox is striving to achieve still greater success.

Many of the best wood engravings in the volume have been engraved by Miss Powell, to whom, as well as to Messrs. Harrison for the great care bestowed in printing, the author's thanks are due.

The author regrets that the book should have been so long out of print, and the publication of the new edition so long delayed. He was anxious to improve it to the utmost of his power and increase its usefulness as a practical work ; and he has, therefore, spared neither time nor trouble, and has refrained from hurrying it through the press, feeling satisfied that time spent in perfecting practical details connected with demonstration, is well employed. For, however some may be inclined to disparage hand work as distinguished from head work, it is certain that no one can become a good microscopical observer, unless he is possessed of considerable manual dexterity, to be acquired only by long practice ; and no work can be higher or more useful than that of assisting to make men original workers in any department of science, and of encouraging original work. Working books by working men will do far more towards these ends than the most brilliant discoveries, and the author believes that earnest men cannot labour more usefully than by endeavouring to make others work.



## PREFACE TO THE SECOND EDITION.

WITH the view of increasing the usefulness of this work, numerous explanatory illustrations have been added in the present edition.

The author, both in the text and in the explanations to the engravings, has endeavoured to restrict himself, as far as possible, to giving hints and directions which may be practically useful to the student while he is at work. All matter that would be merely interesting to the general reader has therefore been altogether omitted. Directions for working cannot well be too explicit and precise, and the more simply they are given, the more useful they are likely to be.

61, Grosvenor Street, January, 1861.

## PREFACE TO THE FIRST EDITION.

AN earnest desire to assist in diffusing a love for microscopical inquiry, not less for the pleasure it affords to the student, than from a conviction of its real utility and increasing practical value in promoting advancement in various branches of art, science, and manufacture,—a wish to simplify, as far as possible, the processes for preparing microscopical specimens, and the methods for demonstrating the anatomy of different textures,—and the belief that many who possess microscopes are deterred from attempting any branch of original investigation solely by the great difficulty they experience in surmounting elementary detail and mere mechanical operations,—are my chief reasons for publishing this elementary course of lectures, which was delivered during the past winter.

It has been thought desirable to append the tables which I have been accustomed to use in my course of practical demonstrations, for the purpose of enabling everyone to practise for himself the most useful branches of manipulation. Each table will occupy the student about two hours.

Subjoined is a list of the apparatus required for microscopical research, much of which is simple and inexpensive. A number has been added to each instrument, by transmitting which to any instrument maker, the observer will be furnished with the apparatus required.

L. S. B.

PATHOLOGICAL LABORATORY,  
27, Carey Street, Lincoln's-inn, June, 1857.



## TO THE READER.

- Some persons may prefer to study the contents of this book by referring, in the first instance, to the plates. The book may, in fact, be "read" in this way. The figures are explained at the lower part of the plate, but under each will be found a reference to the section in which the matter it illustrates is discussed, so that, with a very little trouble, the observer may render himself familiar with most of the instruments and different pieces of apparatus required in microscopical observation. But the author recommends the beginner to "read" the book by practising the tables, commencing at page 249, or if he has not patience for this, he should at least go through the processes in §135, page 73.

## CONTENTS.

### CHAPTER I.

	PAGE
INTRODUCTION.—The Microscope—Simple and Compound Microscopes. OPTICAL PORTION OF THE MICROSCOPE.—The Negative Eye-piece—Positive Eye-piece—The Object-glasses—Spherical and Chromatic Aberration—Angle of aperture—The Mirror. MECHANICAL PORTION OF THE MICROSCOPE.—Adjustments for altering the focus—The Body of the Microscope—The Stage—Diaphragm—Microscope Makers—Students' Microscopes—Necessary Apparatus—Large Microscopes—Binocular Microscopes—Travelling and Dissecting Microscopes—Clinical, Pocket, Travelling, and Class Microscope—Dissecting Microscope	1

### CHAPTER II.

Examination by Reflected Light. Transmitted Light, and Polarized Light. REFLECTED LIGHT.—Different Methods employed—Bull's-eye Condenser—Metallic Side Reflector—Lieberkuhn's—Dark-ground Illumination—Parabolic Illuminator—Annular Condenser. TRANSMITTED LIGHT.—Diaphragm—Achromatic Condenser—Gillett's Condenser. POLARIZED LIGHT.—Polarizer—Analyzer—Iceland Spar—Tourmaline—Iodo-quinine or Herapathite. ILLUMINATION BY ARTIFICIAL LIGHT.—Lamps—Camphine Lamp—Oil Lamps—Argand Lamp—French Moderator Lamps—Gas Lamps—Of the Importance of Protecting the Eyes from the Diffused Light of Lamps. ON DRAWING MICROSCOPICAL SPECIMENS.—Camera Lucida—Steel Disc—Neutral Tint Glass Reflector—Arranging the Light—Of making Drawings which it is intended should be Engraved—Tracing Paper—Retransfer Paper—Wood Blocks—Of obtaining Lithographs of Microscopical Drawings—Drawing on Transfer

Paper—Drawing on the Stone—Of Engraving on Stone— Transfer Paper—Lithographic Ink—Lithographic Stones—On the Importance of Observers Delineating their own Work. ON MEASURING OBJECTS.—Cobweb Micrometer—Test Objects— Nobert's Lines—Jackson's Eye piece Micrometer—Stage Micro- meter—Simple Directions for Measuring Objects—On ascertain- ing the Magnifying Power of Object-glasses—To ascertain the Diameter of an Object—Standards of Measurement—Conversion of Foreign Standards of Measurement—On Finders—On Measuring the Angles of Crystals: Goniometers. ....	19
--	----

## CHAPTER III.

INSTRUMENTS REQUIRED IN MICROSCOPICAL RESEARCH.—Spirit Lamp—Wire Retort Stands—Tripods—Brass Plate—Water Bath. INSTRUMENTS FOR CUTTING THIN SECTIONS OF TISSUES.—Scalpels—Doubled-edge Scalpel—Double-bladed or Valentin's Knife—Scissors—Needles—Forceps—Wooden Forceps. APPARATUS USED FOR EXAMINING OBJECTS IN THE MICROSCOPE.—Plate Glass Slides—Thin Glass—Glass Cells— Watch Glasses. CEMENTS.—Gold Size—Sealing-wax Varnish —Solution of Shell-lac—Bell's Cement—Brunswick Black— Marine Glue—Cement for Attaching India-rubber or Gutta Percha to Glass Slides—Canada Balsam—Of Vessels for Keeping Canada Balsam in—Solution of Canada Balsam— Gum—French Cement Composed of Lime and India-rubber. PRESERVATIVE SOLUTIONS.—Spirit and Water—Glycerine— Thwaites' Fluid—Solution of Naphtha and Creosote—Solution of Naphtha and Water—Solution of Carbolic Acid—Solution of Chromic Acid—Preservative Gelatine—Gelatine and Glycerine —Gum and Glycerine—Goadby's Solution—Burnett's Solution —Solution of Chloride of Calcium—Alum—Arsenious Acid— Arseniuretted Hydrogen—Nitrogen. ....	47
---	----

## CHAPTER IV.

ON THE METHODS OF MAKING CELLS FOR MICROSCOPICAL PREPARATIONS.—Cells for Preserving Microscopical Specimens —Cells for Dry Objects—Paper Cell—Holder for Pressing Objects, &c.—Brunswick Black Cell—Cells made of Tinfoil and Marine Glue—Of Cutting and Grinding Glass—Of Cutting the Thin Glass—Stone or Pewter Slab for Grinding Glass— Cementing Glass together with Marine Glue—Cleaning off Superfluous Glue—Cells made of Thin Glass—Simple Methods of Perforating the Thin Glass—Deeper Glass Cells—Small deep Glass Cells for Injections—Built Glass Cells—Deep Glass Cells made by Bending a Strip of Glass in the Blowpipe Flame —Moulded Glass Cells—Gutta Percha—Troughs for Examining Zoophytes—Animalcule Cages—Round Cells proposed by Dr. Guy. ....	65
---	----

# CONTENTS.

xi

PAGE

## CHAPTER V.

ON EXAMINING OBJECTS IN THE MICROSCOPE.—For Beginners only.—How to Examine an Object in the Microscope. GENERAL CONSIDERATIONS UPON THE STRUCTURE OF TISSUES.—Flesh or Muscular Tissue.—On Demonstrating the Anatomical Peculiarities of Tissues.—The Anatomy of Organs more easily Demonstrated in the Lower Animals than in Man and the Higher Animals.—Of the Time after Death when Tissues should be Examined.—Ciliary Motion. OF PREPARING TISSUES FOR MICROSCOPICAL EXAMINATION.—Of making Minute Dissections.—Dissecting under the Surface of Fluid.—Loaded Corks.—Tablets upon which Dissections may be pinned out.—Of obtaining Thin Sections of Different Textures for Microscopical Examination.—Drying the Tissue before Cutting the Sections.—Hardening the Tissue.—Horn.—Hair.—Making Thin Sections of Bone.—Teeth.—Sections of Wood.—Of Dissecting Tissues under the Microscope with the Aid of the Compressorium.—The Cell or Animalcule Cage. OF THE IMPORTANCE OF EXAMINING OBJECTS IN VARIOUS WAYS.—Appearance of the same Object in AIR, WATER, and CANADA BALSAM, by TRANSMITTED LIGHT, and under the influence of REFLECTED LIGHT and POLARIZED LIGHT. ....	73
---	----

## CHAPTER VI.

ON THE EXAMINATION OF TISSUES AND THEIR PRESERVATION AS PERMANENT OBJECTS. — General Considerations with reference to the Nature of the Medium in which Tissues should be placed for Examination. EXAMINATION AND PRESERVATION OF STRUCTURES IN AIR. EXAMINATION AND PRESERVATION OF SUBSTANCES IN AQUEOUS FLUIDS. EXAMINATION AND PRESERVATION OF SOFT TISSUES.—Muscle.—Villi.—Adipose Tissue.—Nerve Fibres.—Arrangement for pressing down the Thin Glass Cover upon the Preparation while the Brunswick Black is drying.—Examination of Vegetable Tissues.—Of the Circulation in the Cells of Vallisneria.—Anacharis, Anchusa, &c. EXAMINATION AND PRESERVATION OF OBJECTS IN CANADA BALSAM. — Examination of Hard Tissues: Bone. AIR-BUBBLES. OIL-GLOBULES.—Blood-globules.—Fungi. OF THE SEPARATION OF DEPOSITS FROM FLUIDS. — Conical Glasses.—Pipettes.—Removing the Deposit with the Pipettes.—Separation of Deposit when very Small in Quantity.—Examination of Infusoria.—Vorticellæ.—Zoophytes.—On separating the Coarse from the Finer Particles of a Deposit.—Method of obtaining the Silicious Skeletons of Lower Organisms.—Wash-bottle.—Of keeping Preparations in the Cabinet. ....	89
---	----

## CHAPTER VII.

OF INJECTING.—Natural and Artificial Injections.—Transparent and Opaque Injections.—Instruments required for making	
---	--



	PAGE
Injections—Syringe, Pipes, Stop-cocks, Bull's-nose Forceps, Needle for passing the Thread round the Vessel. OF OPAQUE INJECTIONS.—Injecting Cans—Size—Colouring Matters— Vermilion—Chromate of Lead—White Lead—Size of the Particles of the Colouring Matter used. OF TRANSPARENT INJECTIONS.—Injecting with Plain Size—Colouring Matters— Gerlach's Carmine Injecting Fluid.—Advantages of employing Prussian Blue—Composition of the Prussian Blue Fluid for making Transparent Injections—On injecting different Systems of Vessels with Opaque and Transparent Injections—Acid Carmine Fluid—Mercurial Injections. INJECTING THE LOWER ANIMALS.—Mollusca—Insects. OF THE PRACTICAL OPERA- TION OF INJECTION.—Of Injecting the Ducts and Secreting Follicles of Glands—Of preparing Portions of Injected Prepa- rations for Microscopical Examination ....	107

## CHAPTER VIII.

OF CHEMICAL ANALYSIS IN MICROSCOPICAL INVESTIGATION— Instances of the use of Reagents—Preliminary Operations— Reaction—On Filtering—Evaporation and Drying—Incineration— Apparatus—Chemical Microscope—Examining Substances at a High Temperature. REAGENTS AND THEIR ACTIONS.— Alcohol—Ether—Effects of Alcohol and Ether—Nitric Acid— Sulphuric Acid—Hydrochloric Acid—Acetic Acid—Chromic Acid—Effects of Acids upon Organic Structures—Solution of Potash—Solution of Soda—Effects of Alkalies upon Organic Structures—Potash and Soda—Ammonia—Nitrate of Barytes —Nitrate of Silver—Oxalate of Ammonia—Iodine Solutions. OF APPLYING TESTS TO MINUTE QUANTITIES OF MATTER. —Bottles with Capillary Orifices for Tests—Capillary Tubes, with India-rubber tied over the Top—Testing for Carbonates, Phosphates, Sulphates, and Chlorides. OF OBTAINING CRYSTALLINE SUBSTANCE FROM THE FLUID AND TEXTURE OF ORGANISMS.—Formation of Crystals—Influence of various Constituents upon the Crystallization—Separation of Crystals from Non-Crystalline Organic Substances—Examination of Crystals under the Microscope. OF OBTAINING CRYSTALS FOR EXAMINATION.—Preservation of Crystals as Permanent Objects. OF THE HARDENING PROPERTIES OF DIFFERENT CHEMICAL SOLUTIONS.—Mr. Lockhart Clarke's plan of Pre- paring Thin Sections of the Cord—Method of rendering Soft Tissues Hard and Transparent ....	123
---	-----

## CHAPTER IX.

ON TAKING PHOTOGRAPHS OF MICROSCOPIC OBJECTS.—History. APPARATUS.—Camera, with Object-glasses and Stage adapted to it—Mr. Wenham's arrangement without a Camera— Camera applied to the ordinary Microscope—Dr. Maddox's Camera—Arrangement of Drs. Abercrombie and Wilson.	
--	--

	PAGE
ILLUMINATION.—Sunlight—Artificial Light—Of Focussing— Of the Object-glasses—Stereoscopic Photographs—CHEMICAL SOLUTIONS.—Collodion—Nitrate Bath—Of the Developing Solutions—The Fixing Solutions. PRACTICAL MANIPULATION. —Cleaning the Glass Plates—Arranging the Camera— Inserting the Plate—Developing the Image—Varnishing the Plate—Of cleaning Old Plates—Of increasing the Intensity of the Negative. OF PRINTING.—Preparing the Paper and Printing—Toning Solution—Another Toning Solution—Fixing Solution—Of Mounting the Prints—Photographs of Microscopic Objects for the Magic Lantern	149

## CHAPTER X.

NEW METHODS OF PREPARATION.—Of Staining Tissues— Process followed by the Rev. Lord S. G. Osborne—Gerlach's Method of Staining—Thiersch's Method—Thiersch's Lilac Colouring Fluid—Anilin Colours—Blue Colours for Staining— Tannic Acid—Solutions of Nitrate of Silver—Other Metallic Salts—Modification of the foregoing plans. THE AUTHOR'S NEW METHOD OF PREPARATION FOR EXAMINING TISSUES WITH THE HIGHEST POWERS.—Conditions to be fulfilled— Action of Glycerine and Syrup—The Injecting Fluid—The Carminic Acid—Other Colouring Solutions—Glycerine and Acetic Acid—Chemical Reagents in Glycerine—Acetic Acid Syrup—Potash and Soda in Glycerine—Chromic Acid and Bichromate of Potash—Of the advantages of viscid media for the Dissection of Tissues—The practical method of preparing Tissues for examination with the Highest Powers—Of the preparation of Hard Tissues—New Theories relating to Structure and Growth arrived at by this mode of investigation.	189
--	-----

## CHAPTER XI.

OF THE USE OF VERY HIGH MAGNIFYING POWERS.—Objections raised—Of the Highest Magnifying Powers yet made—Of the Covering Glass—Illumination of Objects Magnified by very High Powers—Method of Increasing the Size of the Image without altering the Object-glass—Of Drawing Objects Magnified with very High Powers—The Anatomical Element or Cell and of its Life	215
---	-----

## CHAPTER XII.

OF MAKING AND RECORDING MICROSCOPICAL OBSERVATIONS.— Of drawing Inferences from Observations. FALLACIES TO BE GUARDED AGAINST IN MICROSCOPICAL INVESTIGATION.— Errors of Observation—Of the Commencement and Termination of Tubes—On the Difficulty of seeing Structures from their extreme Transparency—Fibres and Membranes produced Artificially by the Action of Reagents—A Fibrous Appearance	
--	--

produced in Structureless Membranes—Collections of Oil-globules appearing as if within a Cell—On the accidental Presence of extraneous Substances. OF RECORDING MICROSCOPICAL OBSERVATIONS.—Exactness of Description—Of the Importance of making Sketches	....	....	....	PAGE
TABLES FOR PRACTISING THE USE OF THE MICROSCOPE	....	....	....	240
BRITISH AND FOREIGN MICROSCOPE MAKERS	....	....	....	249
PREPARERS OF MICROSCOPIC OBJECTS	....	....	....	263
MATERIALS AND APPARATUS FOR MOUNTING OBJECTS	....	....	....	263
ARTISTS, DRAUGHTSMEN, WOOD ENGRAVERS	....	....	....	263
PRINTERS, LITHOGRAPHIC, AND PHOTO-LITHOGRAPHIC PRINTERS	....	....	....	263
LITHOGRAPHIC STONES, DIAMONDS FOR ENGRAVING, AND APPARATUS USED IN LITHOGRAPHY	....	....	....	263
APPARATUS FOR DRAWING ENGRAVINGS, &c.	....	....	....	263
BRITISH AND FOREIGN WORKS ON THE MICROSCOPE, INCLUDING JOURNALS	....	....	....	264
INDEX	....	....	....	267

## ILLUSTRATIONS.

FRONTISPIECE.—Photographs of pith, itch insects, blood corpuscles, bone, &c. By Dr. Maddox.

Plate I.—Structure of eye-piece and object-glasses of simple and compound microscopes.

Plate II.—Objectives of different angles of aperture. 'Aberration,' spherical, and chromatic. Stage and mirror.

Plate III.—Microscope. By Messrs. Powell and Lealand.

Plate IV.—Student's microscope. Made by Mr. Salmon.

Plate V.—Student's microscope. Designed by Mr. Highley.

Plate VI.—Binocular microscopes. Nacht's microscope for two observers.

Plate VII.—Binocular microscopes. Showing the arrangement of the prisms.

Plate VIII.—Highley's cheap portable and student's microscopes.

Plate IX.—Wenham's original arrangement of the binocular microscope. Travelling microscope.

Plate X.—Microscope as arranged for examining objects in vivaria, and for making dissections.

Plate XI.—View of all the parts of Dr. Beale's pocket microscope.

Plate XII.—Dr. Beale's microscope, as arranged for class demonstration.

Plate XIII.—Clinical or pocket microscope. Student's microscope. Quekett's dissecting microscope.



Plate XIV.—Prof. Quckett's dissecting microscope. The instrument arranged for observation. The same packed up for travelling.

Plate XV.—Illumination by reflected and transmitted light.

Plate XVI.—Lieberkuhn—Condensers, Neutral tint glass reflector, Steel disk, Polariscopes, Camphine lamp.

Plate XVII.—Reflected light with the Lieberkuhn. Achromatic condenser. Parabolic illuminator. Microscope as arranged for drawing objects.

Plate XVIII.—Modes of measuring microscopic objects. Finders.

Plate XIX.—Bailey's universal indicator.

Plate XX.—Goniometer. Microscope for measuring angles of crystals.

Plate XXI.—Retort stand. Spirit lamp. Heating plate. Simple hot water bath. Tripods.

Plate XXII.—Scissors. Scalpels. Valentin's knives. Needles.

Plate XXIII.—Forceps. Section knives. Saws. Glass shades for protecting microscopic objects from dust.

Plate XXIV.—Apparatus for making round cells. Brass ring for cutting glass circles. Shadbolt's apparatus. Diamonds for cutting glass.

Plate XXV.—Strong glass cells of various sizes and shapes.

Plate XXVI.—Thick built glass cells. Deep cells. Cells of peculiar shape.

Plate XXVII.—Portion of the diaphragm of the white mouse, showing elementary muscular fibres, Nerve fibres, and Capillary vessels.

Plate XXVIII.—Yellow fibrous tissue. White fibrous tissue. Muscular fibre. Nerve fibre. Tracheæ. Adipose tissue.

Plate XXIX.—Fat. Small vessel dividing into capillaries. Dissecting under water. Newt. dissected.

Plate XXX.—Loaded Cork. Instrument for cutting thin sections of wood. Compressor.

Plate XXXI.—Carbonate of lime. Different appearances of the same object in different media, by transmitted, reflected, and polarized light. Crystals.

Plate XXXII.—Villi. Epithelium. Altered nerve fibres. Entozoa.

Plate XXXIII.—Vivaria. Fern cases. Branch of anacharis alsinastrium. Cells of anacharis.

Plate XXXIX.—Hair of anchusa. Shells of rare diatoms. Egg of common bug.

Plate XXXV.—Animalecule cage. Heating apparatus. Pressing apparatus. Air Pump.

Plate XXXVI.—Air bubbles. Oil globules. Blood corpuscles. Liver cells. Yeast fungus. Crystals of fatty acids.

Plate XXXVII.—Using the pipette. Wash bottle. Placing on the thin glass cover. Apparatus for exerting a continued pressure upon the thin glass cover.

Plate XXXVIII.—Corks for the injecting pipe. Bull's nose forceps. Injecting can. Syringe.

Plate XXXIX.—Comparative sizes of the particles of some of the different colouring matters used as injections.

Plate XL.—Portion of gall bladder injected. Capillaries of liver. Portion of intestine arranged for injection.

Plate XLI.—Funnel arranged for filtering. Folding of the filtering paper. Test tubes. Rack and drainer. Box with reagents, &c.

Plate XLII.—Dr. J. Lawrence Smith's inverted microscope.

Plate XLIII.—Capillary tubes. Box of capillary-necked bottles. Crystals of creatine.

Plate XLIV.—Crystals of snow.

Plate XLV.—Spinal Cord. Transverse and longitudinal section.

Plate XLVI.—Photographic microscope camera.

Plate XLVII.—Photographic camera, as arranged by Dr. Maddox.

Plate XLVIII.—Photographic plate holder. Sectional views. Lamp with condensing lenses. Pressure frame. Camera adapted to the microscope.

Plate XLIX.—Oxy-hydrogen lantern with gas bag, arranged for throwing microscopic objects upon a screen.

Plate L.—Cells, Animal and vegetable. Formation of starch, fat, &c. Blood corpuscles, their development, growth, crystallization, &c.

Plate LI.—Blood crystals. Blood corpuscles. Mucus corpuscle. Bacteria.

Plate LII.—Mildew. Cells of sea weed.

Plate LIII.—Portion of myohyoid muscle of the *Hyla arborea* × 1700.

Plate LIV.—Ganglion cell with straight and spiral fibres.

Plate LV.—Ganglion cells, showing mode of development.

Plate LVI.—Extraneous matters: Potato starch. Fibres of deal. Fragments of hair. Portions of feather. Spiral vessels of tea leaf, &c.

Instrument for containing glycerine and other preservative fluids, p. 214.

The same object magnified by different magnifying powers, p. 218.

# THE MICROSCOPE

AND

## MICROSCOPICAL MANIPULATION.

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### CHAPTER I.

INTRODUCTION.—*The Microscope—Simple and Compound Microscopes.* OPTICAL PORTION OF THE MICROSCOPE.—*The Negative Eye-piece—Positive Eye-piece—The Object-glasses—Spherical and Chromatic Aberration—Angle of Aperture—The Mirror.* MECHANICAL PORTION OF THE MICROSCOPE.—*Adjustments for altering the Focus—The Body of the Microscope—The Stage—Diaphragm—Microscope Makers—Students' Microscopes—Necessary Apparatus—Large Microscopes—Binocular Microscopes—Travelling and Dissecting Microscopes—Clinical, Pocket, Travelling, and Class Microscope—Dissecting Microscope.*

**1. Introduction.**—The course of instruction which I am about to commence will embrace the consideration of many subjects of a strictly practical character, and although it may be found devoid of that interest which necessarily attends the description of the structure of living beings, or the theoretical speculations upon the causes of vital phenomena, I trust it will prove practically useful to those who desire to prosecute microscopical research.

My aim will be to describe the mode of examining different objects in the microscope, the best methods of displaying their structure, and the manner in which they may be preserved permanently. How best to demonstrate the peculiarities of a structure is a question often asked by the microscopist, and it is an important one, for upon the method employed very much

depends. The success which attends our efforts in this field of research is, I believe, in great measure dependent upon our knowledge of the various methods which experience has shown to be advantageous for rendering the anatomical peculiarities of a texture clear and distinct, and it will be found that the most important new facts which have lately been added to science have been discovered by men who have paid the greatest attention to the action of various re-agents, and have devised new and ingenious modes of investigation.

From my position as a teacher of physiology and morbid anatomy and of clinical medicine in a large medical school, I have been naturally led to direct my attention chiefly to those branches of microscopical investigation which belong more particularly to my own department, or which bear directly upon the investigation and treatment of disease; but in this work I shall exclude everything of a strictly professional character. I shall allude only to those processes applicable to general microscopical research, and to the investigation of animal and vegetable tissues.

Many little points to which I shall have to refer may perhaps be stigmatized as merely mechanical; others may be regarded as belonging rather to the province of the chemist than to the microscopical observer; and not a few will perhaps seem to some readers unimportant and hardly worthy of attention.

Some may consider such matters of manipulative detail to be out of the province, or even beneath the notice, of a scientific observer or a medical practitioner; but those who feel the reality and the usefulness of thorough work will not think this. No man ever did perform real work until he had himself mastered minute practical details. Every one who has experienced the happiness of devoting himself to original research naturally desires to encourage others in the same course; and how can this be done better than by showing as clearly as is possible how good work may be carried out?

There are few matters upon which more misapprehension exists among young men than this: how to master elementary practical details, by which alone real success in the higher branches of work can be attained. Not a few express contempt for elementary work and mechanical skill, by the aid of which alone can any one hope to add to existing knowledge. This false notion has not been discountenanced by teachers in the firm



manner in which it should have been met. In this particular branch of inquiry, for example, the importance of injection and of preparing specimens and other practical work has been very much underrated, and in some cases even contemned as useless waste of time. It is the very grammar of the subject, which must be mastered and mastered thoroughly. The number of original observers emanating from our schools will vary in proportion to the encouragement afforded to this kind of work.

I feel so very strongly that success in microscopical as well as chemical inquiries is connected with a readiness in surmounting comparatively small difficulties and with the possession of mechanical dexterity, that I feel it a duty to dwell somewhat on the subject; and I should be doing a great injustice to my pupils if I did not instruct them in microscopical manipulation, and endeavour to facilitate, as far as possible, the performance of those operations which are essential to the successful demonstration of the structure of textures under the microscope. These are questions not beneath the consideration of any one who takes a real interest in studying the structure of the different organisms by which he is surrounded,—and it is the same in this as in other branches of inquiry, that he who is most fully conversant with elementary detail will be the most successful in the consideration of the higher and more abstruse problems, while he will feel a real love for his work, which is denied to the mere superficial inquirer.

To endeavour to discover new methods of investigation appears to me to be one of the most important duties of every observer. To communicate these to his pupils must be the desire of every teacher of any branch of natural science.

I am strongly of opinion that it is more necessary than ever that we should teach as much as possible by the eye. In teaching any branch of natural science the *demonstration* should be combined with *oral* teaching. The student should *see* what is described; and where it is not possible for the teacher to exhibit illustrative specimens, good models, drawings, and explanatory diagrams should be supplied. It is the duty of every teacher to study how to communicate knowledge *most easily* and *most clearly* and to save the student as much time as possible, for it is not likely that the amount of work which is required by the various examining boards will be reduced, nor indeed is it desirable that it should be. It is therefore incumbent upon teachers to facili-



idea of structure, which it is impossible for him to reach by reading or from mere description with the aid of diagrams.

By describing the results of the investigations of others, the teacher is enabled to diffuse knowledge. By detailing the conclusions which he has arrived at from our own investigations, each master adds his mite to the gradually increasing stock of information. By impressing strongly on his pupils the nature of the steps by which conclusions in scientific inquiries are at length arrived at, and by describing to them the methods employed in investigations, the teacher not only encourages his pupils to become original *observers*, and to imitate themselves, but he places them in a position to continue the researches at a point where they have been abandoned by preceding observers.

Microscopical inquiry may be undertaken by persons in almost any position. The numerous cheap and excellent instruments which have lately been made by many English makers have largely contributed to diffuse a knowledge of minute nature. The annually increasing sale of instruments of all kinds shows how popular this branch of inquiry is becoming; yet it must be confessed that the additions to scientific knowledge by no means so great, as a consideration of these circumstances would have led one to anticipate, and although there are many instruments, I fear it must be confessed that the number of observers is comparatively few.

The opinion, that it is only necessary to place an object under the field of the microscope in order to make out its structure, is too prevalent. To this erroneous idea much of the

necessary accessory apparatus. The many excellent books in our own language render it unnecessary for me to occupy time in a minute description of the parts of which the instrument is composed, and to these works I must refer for information on this head.\* At the same time it will be well for me to allude in general terms, and very cursorily, to certain points which every good microscope should possess, and to refer very briefly to the general form of instrument required by the student.

*The Compound Microscope* is the only one now used for microscopical research. Until those great improvements in the mode of making the glasses, now universally employed, had been introduced by the successful labours of Mr. Lister, Mr. Ross, and others, the compound microscope was a very imperfect instrument, and even up to the present century the simple microscope, as employed by Leeuwenhoek, and improved by Wollaston and others, possessed many advantages over its more complex but imperfect rival. I shall not attempt to explain those beautiful optical principles upon which the value of the microscope, as an instrument for minute research, depends. There are, however, several terms in constant use to which I shall have occasion to allude which it seems to me desirable to explain briefly.

**3. Simple and Compound Microscopes.**—In the *simple microscope* (Fig. 1, Plate I) the magnified image of the object passes at once to the eye of the observer.

In the *compound microscope* (Fig. 2) the object is magnified in the first instance by the *object-glass*, c, and brought to a focus within the tube, as represented at a, in the diagram. This magnified image is again magnified by the *eye-piece*, b. The image is of course inverted, but this inconvenience may be obviated by causing it to pass through another set of lenses inserted in the tube of the microscope, and termed the *erector*. The magnifying power, then, of the compound microscope may be increased either by increasing the power of the *object-glass* or that of the *eye-piece*, or by increasing the distance between the *object-glass* and the *eye-piece*. It must be borne in mind, however, that in increasing the power of the *eye-piece* we do not magnify the *object* itself in a greater degree, but simply increase the size of the *image* of the object formed by the *object-glass*. Any imperfections which may

\* For list of works see end of the volume.

exist in the object-glass are thus greatly increased. Hence we should never work with deep eye-pieces, but when we wish to magnify an object to a greater degree, we should adapt a higher power to the instrument. Information upon obtaining very high powers will be given in the last chapter.

In glancing cursorily at the structure of the microscope, it will be convenient for me to allude in the first place to the *optical portion* of the instrument, and secondly to the *mechanical appliances* for moving the object, altering the focus, &c. The *optical portion* includes the eye-piece, object-glass, and the mirror from which the light is reflected so as to pass through the object.

#### OPTICAL PORTION OF THE MICROSCOPE.

4. **Negative Eye-piece.**—The *eye-piece* in ordinary use is the *negative* or *Hughenian* eye-piece (Fig. 3, Plate I). It consists of two plano-convex glasses, the flat surfaces of each being directed upwards. The one nearest the eye of the observer is the *eye-glass*, and the one at the greater distance the *field-glass*.

5. **The Positive Eye-piece**, of Ramsden (Fig. 4), is only used in those cases in which it is necessary to see distinctly some object in the eye-piece, as an instrument for measuring, at the same time that the object itself is in focus. In the latter eye-piece the convex surfaces of each of the two glasses are directed towards each other as represented in this diagram.

Kelner's eye-piece is made like the negative eye-piece, but the eye-glass is an achromatic combination. At the suggestion of Mr. Brooke I have lately used this eye-piece as a condenser with the best results.

6. **Object-glasses.**—The *object-glasses* (Fig. 5, Plate I) used in the best instruments are of English manufacture, but some of those furnished with the cheap microscopes are made on the continent, and are much less expensive. The defining power of many of these foreign objectives is very good, and they are admirably adapted for all ordinary work—they vary in price from ten to thirty shillings, but a good English quarter of an inch glass cannot be purchased for less than five pounds.

The two most useful object-glasses for the student are the *quarter of an inch* which should magnify from 200 to 220

PLATE I.

Fig. 2.



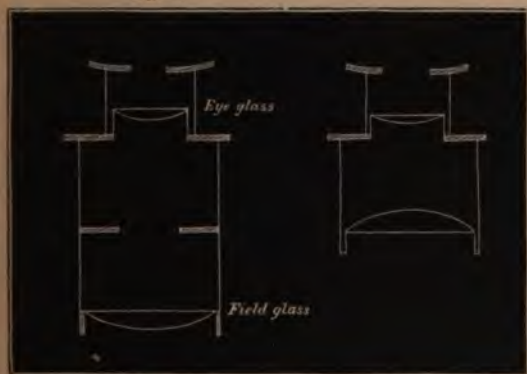
§ 3.

Fig. 1.



§ 3.

Fig. 3.



§ 4.

§ 5.

Fig. 5.



§ 6.

Fig. 1. Illustrates the manner in which an object is magnified by the simple microscope or by a lens.

Fig. 2. Diagram of compound microscope. A. Point where the object is brought to a focus by the object-glass (C). The image formed at this point is magnified again by the eye-piece B.

Fig. 3. Negative or Huyghenian eye-piece.

Fig. 4. Positive eye-piece invented by Ramsden.

Fig. 5. Compound glasses of an achromatic object-glass.

[To face page 6.]





diameters, and the *inch* which should magnify from 30 to 40 diameters. The definition of these glasses should be good, and they should transmit plenty of light. Any lines in a structure examined by them should appear sharp and distinct. The whole field should be perfectly flat, and every part of it in focus at the same time. The field should not be too small, and there should be no coloured rings round any objects subjected to examination.

Some object glasses are now made so that the object must be viewed through a thin stratum of water placed upon the surface of the covering glass (*à immersion*). Hartnach's powers are among the best made upon this principle. Mr. Brooke has well observed that by this plan the object is much more highly illuminated, because rays are transmitted, which in the ordinary process of examination are reflected from the lower surface of the object-glass.

**7. Spherical and Chromatic Aberration.**—A glass is said to be uncorrected for *spherical aberration* when objects at the circumference of the field are not in focus at the same time as those in its centre (Fig. 7, Plate II), and it is not corrected for *chromatic aberration* if there are coloured fringes around any objects subjected to examination by it (Fig. 8). The *defining power* of an object-glass will be very imperfect if it be not properly corrected for spherical and chromatic aberration.

**8. Angle of Aperture.**—For ordinary work it will be found inconvenient if the object-glass, when in focus, comes too close to the object. This is a defect in glasses having a high angle of aperture. The *angle of aperture* is the angle made by two lines from opposite sides of the aperture of the object-glass with the point of focus of the lens. The angle B A B in Fig. 6 is the angle of aperture. Glasses with a high angle of aperture admit much light, and define many structures of an exceedingly delicate nature, which look confused when examined by ordinary powers. For general work I recommend glasses with an angle of not more than from 50 to 100 degrees.

Mr. Ross has lately made glasses having an angle of 170 degrees, which are valuable for investigations upon many very delicate and thin structures, such as the diatomaceæ; but such powers are not well adapted for ordinary work. The importance of arranging the object very carefully and the necessity of paying great attention to the illumination, render these glasses

inconvenient for general observation. The *penetrating power* of glasses with a low angle is much greater than in those of a high angle of aperture, so that exact focussing is much more important in the latter than in the former.

The refraction produced by the passage of the light through the thin glass covering the object varies according to its thickness, and it has been found necessary to render the higher powers capable of being adapted to this variable refraction. It is especially necessary in glasses of high angle of aperture, and is effected by altering the distance between the second and third pair of glasses. An engraved line shows the point to which the lens should be screwed up when adapted for *uncovered objects*, and another corresponds to its position for covered objects. In order to adjust the object-glass, it is first arranged for an uncovered object; then any object covered with thin glass is brought into focus by moving the body of the microscope; next, the ring which carries the third lens is screwed round until any particles of dust upon the upper surface of the glass are brought into focus. The glass is then "*corrected*" for examining the covered object which may be brought into focus.

9. **The Mirror.** (Fig. 10, Plate II) should be capable of movement upon an upright beneath the stage, so that it may be arranged near to, or at a distance from, the object, and it should be capable of being inclined at any angle, so that rays of light may be reflected from it and made to pass directly through the object in straight lines, or thrown upon it in a very oblique direction. The mirror should be of full size, one surface quite plane and the other concave, so that a strong light may be condensed upon the object when required.

#### MECHANICAL PORTION OF THE MICROSCOPE.

In directing attention to the mechanical arrangements of the microscope, I must say a few words upon the adjustments for altering the focus, the body of the instrument, and the stage.

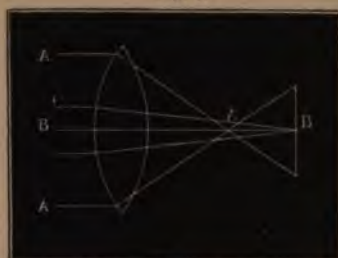
10. **Adjustments for altering the Focus.**—The ordinary movement is obtained by the rack and pinion. In some the body is moved by the fingers alone, and is arranged to slide in a tube like a telescope. In the instruments of Mr. Ladd the requisite

Fig. 6.



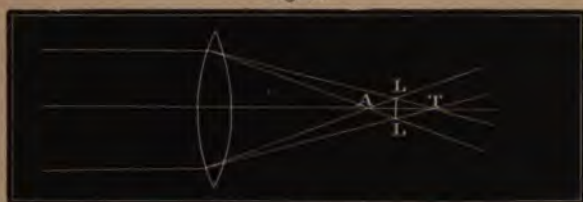
§ 7.

Fig. 7.



§ 7.

Fig. 8.



§ 7.

Fig. 9.



§ 12.

Fig. 10.



§ 9.

- Fig. 6. Objective, with low angle of aperture  $BAB$ . Another with high angle of aperture  $BAB$ .  
 Fig. 7. To illustrate "spherical aberration." The rays  $AA$  being more refracted than those near the centre  $B$ , are brought to a focus nearer the lens.  
 Fig. 8. To illustrate "chromatic aberration." The violet and blue rays being most refrangible are brought to a focus,  $A$ , nearer the lens than the red rays,  $T$ , which are the least refrangible of the rays of the spectrum. Any object placed at  $L$  would exhibit coloured fringes.  
 Fig. 9. Stage of student's microscope, showing diaphragm (Fig. 13) placed beneath. From  $a$  to  $b$  should not be less than two inches (Fig. 12).  
 Fig. 10. Mirror.

[To face page 8.]





motion is obtained by the ordinary milled head, while delicate focussing is carried out by a lever. The movement is by a chain instead of rack and pinion.

Besides these coarse adjustments, however, every microscope should be provided with a more delicate motion for altering the focus when high powers are employed. The *fine adjustment* is differently arranged in detail in various instruments, but is effected by turning a screw having a very fine thread. The movement of Mr. Ladd's chain is so regular and delicate as to supersede the necessity of a fine adjustment. Mr. Highley has adopted the chain movement.

**11. The Body of the Microscope.**—The instrument should be perfectly steady, whether the body be inclined or arranged in a vertical position; and not the slightest lateral movement or vibration should be communicated to the body of the microscope when the focus is altered by turning either of the adjustment screws. The base or foot should be sufficiently heavy to give steadiness, and should be placed upon three small feet.

The body ought to be provided with a joint by which it may be inclined or placed in a horizontal position, which is required when drawings are made with the camera, or when objects are measured by the aid of this instrument. Another advantage gained by this moveable joint is that the muscles of the neck do not become so tired when the body of the microscope is inclined as when the head has to be bent, for several hours at a time, over an instrument standing upright. The larger the microscope may be, the more necessary is this joint for the comfort of the observer; and as it in no way impairs the steadiness of the instrument, and only adds a few shillings to the expense, I recommend every one, in the choice of a microscope, to select an instrument which may be placed in a vertical, inclined, or horizontal position. The existence of this joint can do no harm, and if the observer never intends to incline his microscope, it is at least desirable that such an alteration in its position should be possible.

**12. The Stage** should be sufficiently large to admit either edge of a glass-slide, two inches in diameter, to be brought under the object-glass (Fig. 9, Plate II). The stage of the microscopes of Næth, Oberhäuser, and some other foreign

makers is too small. The distance from the upright pillar *a* (Fig. 9) to the centre of the object-glass on a level with *b* should not be less than two inches.

13. **Diaphragm.**—Beneath the stage a circular diaphragm with holes in it of several different sizes should be so arranged that it can be made to revolve without difficulty and any hole brought under the object; a catch is of great advantage in placing the hole in the centre of the field (*see* Fig. 9).

14. **Microscope Makers.**—The great number of different microscopes and the excellent workmanship employed in their construction render it a difficult as well as a delicate task for a teacher to recommend any special one to his pupils. Although many of the instruments which I have used are exceedingly good, I doubt not that there are others, which I have never had an opportunity of testing, quite as good in every respect. In the next *section* the instruments of several makers are alluded to, and the names and addresses of the principal English and Foreign microscope makers will be found at the end of the volume.

It is due to those makers who have taken the lead in the manufacture of cheap microscopes that their instruments should be specially referred to. By *cheap microscopes* I mean instruments which, with two powers, an inch and a quarter-inch, can be purchased for about five pounds.

15. **Students' Microscopes.**—Mr. Salmon, Mr. Highley, and Mr. Matthews were, so far as I know, the first makers in London who brought out a really good, cheap, practical instrument, furnished with foreign object-glasses. Mr. Highley's first microscope is represented in Plate V; Mr. Salmon's student's microscope is represented in Plate IV; Mr. Highley's recent pattern in Figs. 41 and 42, Plate XIII; Messrs. Murray and Heath's new microscope (7*L*) is a very good and most convenient instrument.

I would strongly recommend all who are about to purchase a student's microscope to examine the instruments of these makers. I can also recommend the new microscope of Messrs. Smith and Beck, which costs five pounds.

The microscope made by Mr. Field, of Birmingham, which

Fig. 11.



Microscope of Messrs. Powell and Lealand, adapted for all purposes, which folds up and packs in a small flat case. § 15.

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Fig. 19.



Mr. Salmon's student's microscope.  $\frac{1}{4}$  15.

[To follow Plate III.]





Fig. 13.



Student's microscope, designed by Mr. Highley, on a stand, so that it may be readily covered with a glass shade. § 15.

[To follow Plate IV].





gained the medal at the Society of Arts, is, for the price, an exceedingly good instrument. It is provided with two eyepieces, two object-glasses (magnifying from 25 to 200 diameters), bull's-eye condenser forceps, and a live box, and, packed in mahogany case with this apparatus complete, costs only three guineas.

Those who wish for a microscope as perfect as can be made in the present day, I should advise to look at the beautiful instruments of Powell and Lealand, Ross, and Smith and Beck. In alluding specially to these instruments, I wish it to be distinctly understood that I do not in any way disparage the work of other and less celebrated makers. As I have had very great experience of the instruments of Messrs. Powell and Lealand, I feel it right to state that I have always found their work most excellent. These makers have done much to perfect the compound microscope, and they have produced the highest and most perfect object-glasses yet made in Europe. Messrs. Powell and Lealand's microscope, which folds up in a very small space, is represented in Plate III.

In choosing a microscope, the following requirements should be borne in mind:—With reference to the optical part,—the *inch object-glass* should magnify not less than 30 diameters, and the *quarter* not less than 200, when the *shallow eye-piece* is applied. The *field* should be well lighted, and the lines of delicate objects submitted to examination should be sharp and well defined, without coloured fringes when placed in the centre or at the circumference of the field. The mirror should be large (at least two inches in diameter), one side plane, the other concave, and it should be adapted to the body of the microscope in such a manner that the distance from the object may be increased or diminished, while it is also necessary that it possesses lateral movement, in order that very oblique rays of light may be made to impinge upon the object.

With regard to the mechanical portion of the microscope, the adjustments should work smoothly, and an object placed in the field for examination should not appear to move or vibrate when the screws are turned. The body should be provided with a joint, so that it may be inclined or placed quite horizontally. The stage should be at least *three inches in length* by *two and a half in width*, and there should be a distance of at least an inch and a half from the centre of the opening in the stage over which

the slide is placed, to the upright body. The motion of the slide upon the stage, and all other movements and adjustments, should be smooth and even, without any tendency to a jerking or irregular action.

**16. Necessary Apparatus.**—Every student's microscope should be provided with a *neutral tint glass reflector for drawing and measuring objects*, a *diaphragm*, to the under part of which is fitted a tube to receive an *achromatic condenser*, or *polarizing apparatus*: a *bull's-eye condenser*, one *shallow eye-piece*, and two powers—a *low one, magnifying from 20 to 40 diameters*, and a *quarter of an inch which magnifies at least 180 diameters*, a *stage micrometer* (§62), a *Maltwood's finder* (§67), and an *animalcule cage* (§133).

These instruments should be conveniently packed in the case with the microscope. The polarizing apparatus and the achromatic condenser are not absolutely necessary for a beginner and can be purchased afterwards. The cost of the microscope without these last instruments, but including the other apparatus mentioned, in a well-made case, should not be more than six pounds; and if the microscope be mounted upon a cast-iron foot instead of a brass one, it may be obtained for about a pound less, without its practical utility being in any way impaired.

**17. Large Microscopes.**—The large expensive microscopes are provided with every instrument which modern science has placed at the disposal of the observer. For delicate investigations many of these are invaluable, but for ordinary work they are not necessary, and their expense is so great as to place them beyond the reach of the great majority of observers. Very expensive and delicate instruments are required so seldom in ordinary work that most observers will be able to examine any special preparations under the instrument of a friend, whenever such very minute examination is necessary. The members of the Microscopical Society have the advantage of using under certain regulations most beautiful instruments provided with very high powers. A very complete instrument has been liberally placed at the disposal of the society by Mr. Ross. These microscopes are now arranged ready for work at the rooms used by the Society at King's College from 6 to 8 o'clock on each evening the Society meets.

Fig. 15.



§ 18.

Fig. 16.



§ 18.

Fig. 17.

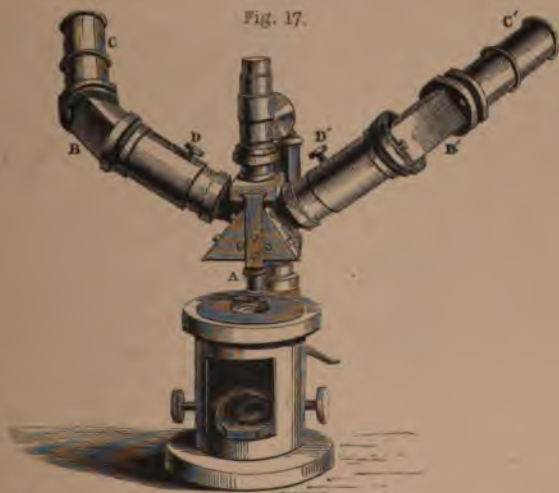


Fig. 15. Nachet's binocular microscope.

Fig. 16. Binocular microscope as recently arranged by Mr. Wenham, and now generally adopted.

Fig. 17. M. Nachet's microscope to enable two observers to examine an object at the same time.

[To face page 12.]



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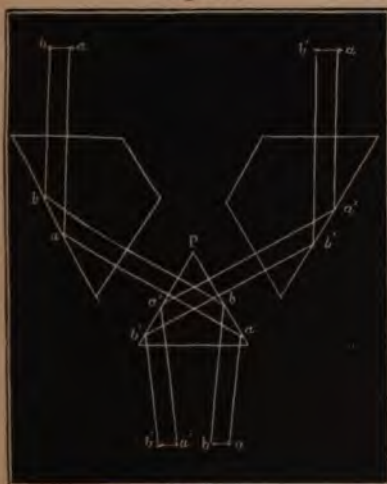


Fig. 18.



§ 18.

Fig. 19.



§ 18.

Fig. 18. Arrangement of Professor Riddell's binocular microscope.  
 Fig. 19. Arrangement of the prisms in Nachet's binocular microscope.  
 Fig. 20. Horizontal binocular microscope of Mr. Wenham.

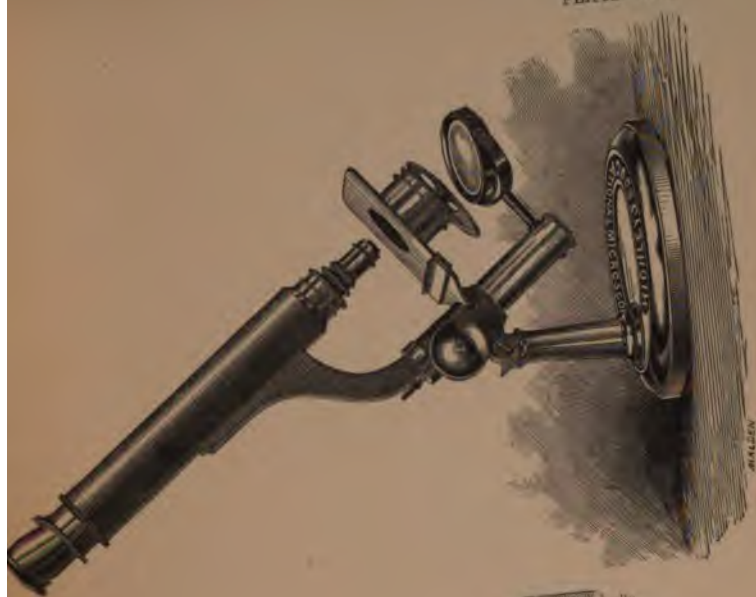
Fig. 20.



[To follow Plate VI.]



PLATE VIII.



Very cheap portable microscope and student's microscope, lately arranged by Mr. Highley. § 20.  
[To follow Plate VII.]





18. **Binocular Microscope.**—M. Nacet's instrument and Mr. Wenham's perfected binocular is represented in Plate VI, and in Plate VII drawings of other binocular microscopes are given. Mr. Wenham has succeeded in producing the most perfect arrangement of this kind. The first plan he adopted is represented in Plate IX, Fig. 21; but the new method last suggested by him, and now adopted by all microscope makers in this country is seen in Plate VI, Fig. 16.

The binocular is applicable to almost every kind of microscopical research, but it is not necessary for the working student, and where very high powers are required, the appearances are not so perfect as could be desired. I cannot recommend those who wish to work at microscopical investigation generally, to provide themselves with a binocular microscope only. In practice it will be found most convenient to work with a very simple instrument. The binocular should be a separate microscope altogether, or it should be possible to remove the binocular tube from the body of the microscope and substitute for it a simple tube. Excellent and cheap binocular microscopes (about 10*l*.) are made by Messrs. Crouch, Messrs. Murray and Heath, and other makers. (*See* the list of makers at the end of the volume).

19. **Travelling Microscopes.**—*Mr. Warington's Arrangement.*—For travelling, especially for sea-side work, it will be convenient to be provided with a microscope which can be packed in a smaller compass than the instruments before alluded to.

Mr. Warington, some time since, designed a very simple microscope for travelling purposes. The stand consists of two flat pieces of oak, fitted at right angles to each other by means of pegs. The stage is inserted into the longer one, to the top of which the body of the microscope is adapted by means of a clamp, in which a horizontal bar carrying the body can be moved backwards and forwards. This instrument can be arranged in an upright or standing position, and by means of the clamp the body can be attached to a table, so that living objects in upright glasses can be subjected to examination. In its present form, however, the instrument is not so steady as could be wished, but by a slight modification in its structure it could probably be made more so.

**20. Travelling, Dissecting, and Vivarium Microscope.**—Another simple form of travelling microscope is described by me in the fourth volume of the Transactions of the Microscopical Society (page 13). This instrument is made entirely of tubes, is very steady, and can be used in any position; it is represented in various positions in Plates IX and X. It makes an excellent microscope for dissecting, and the alteration of focus is effected very rapidly by means of a knee lever, which was kindly made for me by Mr. Becker, instead of a screw.

This microscope takes to pieces and can be packed in a very small case. Its structure is so simple that it cannot easily get out of order. The legs of the tripod stand have been made with hinged joints by Mr. Matthews, which diminishes the bulk of the instrument, when packed up, to a still greater degree. The price, however, of this microscope is 5*l*. (although I believe it might be well made for considerably less), while that of Mr. Warington can be purchased for half that sum.

Mr. Highley has suggested a very cheap form of travelling microscope which is also strong. This is described in the Microscopical Journal, Vol. IV, page 278. It is represented in Plate VIII.

**21. Clinical, Pocket, Travelling, and Class Microscope.**—Under this head I propose to describe an instrument devised by me some years since which I have found very useful for general observation, in the field, and also for medical work, and for class demonstration.

*The Microscope.*—Like some other instruments which have from time to time been proposed, this microscope is composed of draw-tubes like a telescope; but the arrangement of the stage, and the plan adopted for moving the slide when different parts of the object are submitted to examination, differ entirely, as far as I am aware, from those hitherto adopted. The instrument consists of three tubes, *a*, *b*, *c* (Fig. 26, Plate XI); *a* carries the eye-piece, is four and a-half inches long, and slides in *b*, which is of the same length, but only slides up to its centre in the outer tube *c*. Tube *b* carries the object-glass. The tube *c* can be fixed by aid of a screw ring *d*, at any height, according to the focal length of the object-glass. This arrangement prevents the risk of the object-glass being forced through the preparation while being focussed. At the lower part of the body is a screw clamp

Fig. 21.



Fig. 22.

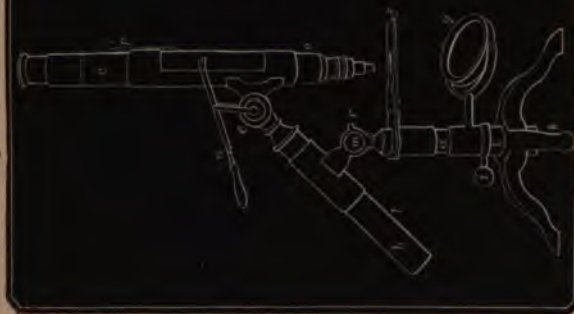


Fig. 23.

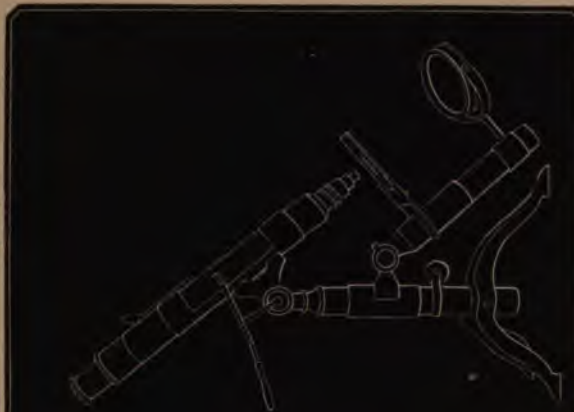


Fig. 21. Mr. Wenham's original arrangement of the binocular microscope. § 18.  
 Figs. 22 and 23. Travelling microscope. *a*. Telescopic stem of the microscope made of brass tubes an inch in diameter. *b*. Horizontal arm of ditto.  
*c*. Body. *d*. Hinge joint. *e*. Tube with clamp screw. *f*. In which the lower part of the stem slides. *g*. Lower part of  
 stem, to which the mirror can be adapted. *h*. Ridge which prevents the horizontal bar from turning round. *m*. Hinge joint. *n*. Knee lever  
 for adjustment. *o*. Fine adjustment screw. *p*. Fin which prevents the body being forced lower than the focus of the object-glass. § 20.





(Fig. 28) for fixing the preparation in any particular position, and an aperture for throwing the light on opaque objects. The preparation is kept in contact with the flat surface below by a spring, which allows the requisite movements to be made with the hand (Fig. 30).

That part of the object which it is desired to examine can easily be placed opposite the object-glass if the instrument is inverted. Next, the focus is obtained by a screwing movement of the tube *b*; and if it be desired to examine any other parts of the object, this is easily effected by moving the slide with one hand, while the instrument is firmly grasped with the other. Delicate focussing is effected by drawing the tube *a* up and down. By this movement the distance between the eye-piece and object-glass is altered.

Any object-glass may be used with this instrument. I have adapted various powers, from a *three-inch*, magnifying *fifteen diameters*, to a *twelfth*, magnifying *seven hundred diameters*, and I feel sure that even higher powers may be used.

In the examination of transparent objects, ordinary daylight or the direct light of a lamp may be used; or, if more convenient, the light may be reflected from a sheet of white paper, or from a small mirror inclined at the proper angle, and placed on the table.

In examining objects by reflected light, sufficient illumination is obtained from an ordinary wax candle placed at a short distance from the aperture, just above the object. But the most beautiful effects result from the use of the Lieberkuhn with direct light.

The slide, as has been stated, is kept in contact with the lower part of the instrument, which I have called the stage, by a spring which is therefore made to press on the *back of the slide*. On the other side of the stage a little screw and clamp are placed so that the specimen may be fixed in any position that may be desired (Figs. 28 and 29).

In using this microscope, the slide with the object to be examined is placed upon the stage, the thin glass being upwards towards the object-glass, while the spring is made to press upon the *under* surface of the slide. The little screw is removed. The slide may now be moved in every position, and any particular object to be examined can readily be placed exactly under the object-glass. Tube *a* is withdrawn about two-thirds

of its length. The tube *c* being firmly held with the left hand, *b* is grasped with the right, and with a screwing motion the object-glass is brought to its proper focus. The specimen having been fixed with the little clamp, and the tube fixed in its position by screwing down the ring fitted on tube *c*, the instrument may be passed round a class. This microscope seems to be well suited for field-work and especially for botanical purposes. It is not heavy, and, including the powers and an animalcule cage, will easily pack into a tube or case six and a-half inches long and two inches in diameter. I constantly use it in clinical teaching. Various deposits, specimens of sputum, &c., may be examined by the patient's bedside, and their characters demonstrated to the class. I think that a most efficient instrument of this kind could be made for 30s., or, with one low power only, even for a guinea.

The instrument is made by Messrs. Powell and Lealand, by Mr. Matthews, Mr. Highley, and other makers.

*The Stand.*—The arrangement of the stand will be at once understood by reference to Fig. 35, Plate XII. The structure of the lamp is represented in Fig. 33, Plate XI. It is an ordinary oil lamp with a diaphragm, just level with the wick, in order to cause a powerful current of air around the flame. By this means all flickering is prevented, and the instrument may be moved about without fear of the light being blown out. The diaphragm is made of a plate of mica, and the same substance is placed over the aperture in the chimney *h*. The lamp is made to slide in the grooves marked *b, g*, Fig. 35, Plate XII, and it is fixed at the proper distance from the object by the screw, Fig. 33 *l*. When required for reflected light, it is placed in the groove marked *c*, Fig. 35. A good modification of the lamp has lately been made for me by Mr. Highley which possesses some advantages over the one figured. It is probable that this may still be somewhat modified. In the last arrangement the lamp is made to slide on a horizontal bar which turns on a pivot, so that the position for reflected light is easily secured (Plate XIII, Fig. 40). A mirror is employed by daytime, and slides in the same groove, or upon the same rod, as the lamp.

The mirror, achromatic condenser, polariscope, and drawing apparatus can all be readily adapted to this instrument, and from its simplicity it will probably be found very convenient for photographic purposes. The microscope, without powers, can be



Fig. 27.



§ 21.

Fig. 26.



§ 21.

Fig. 28.



§ 21.

Fig. 29.



§ 21.

Fig. 31.



§ 21.

Fig. 30.



§ 21.

Fig. 32.



§ 21.

Fig. 33.



§ 21.

Fig. 34.



§ 21.

Fig. 26. Pocket or clinical microscope. Half the real size. Fig. 27. Represents the arrangement by which diaphragm, mirror, and condenser may be adapted to the pocket microscope. Fig. 28. Screw with an arm, for fixing the specimens, *c*, Fig. 26. Fig. 29. Undersurface of the body, or stage of the pocket microscope. Fig. 30. The stage, side view, showing position of the spring. Fig. 31. Mirror employed for examining objects by transmitted light. Fig. 32. Sectional view of cell for examining deposits in fluid, infusoria, &c. Fig. 33. Lamp, sectional view, for illuminating objects examined by this microscope, with screw, *l*, to fix it. See Fig. 35. Fig. 34. Mirror, with screw to fit into slide, Fig. 25.

[To face page 16.]



Fig. 35.



§ 21.

Fig. 36.



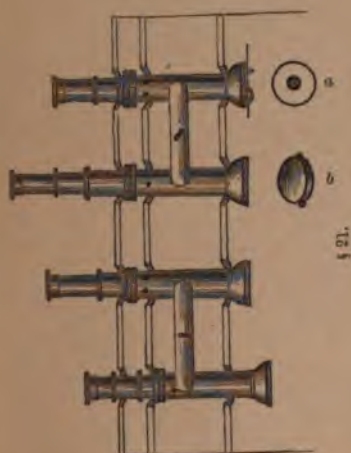
§ 21.

Fig. 37.



§ 21.

Fig. 38.



§ 21.



§ 21.

Fig. 35. Microscope for class demonstration. Fig. 36. Octagonal box, with eight microscopes, illuminated by one lamp in the centre. Fig. 37. Section of 36, showing relative positions of microscopes and lamp, mode of fixing, &c. Fig. 38. Another arrangement for mounting four of the simple microscopes. Section. a, lamp, b, mirror for daylight. Fig. 39. Front view of the same.

[To follow Plate XI.]



Fig. 40.



§ 21.

Fig. 41.



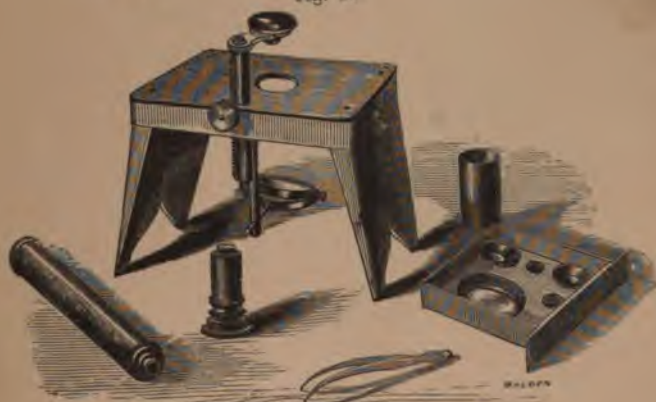
§ 17.

Fig. 42.



§ 15.

Fig. 43.



§ 22.

Fig. 40. Clinical microscope arranged for class demonstration, with stand.

Fig. 41. Mr. Highley's best microscope.

Fig. 42. Mr. Highley's student's microscope, with two powers. Four and five guineas.

Fig. 43. Quekett's dissecting microscope with apparatus.

[To follow Plate XII.]



purchased for twenty-five shillings, and with the stand it will probably cost not more than three pounds.

In using the instrument, the object is first adjusted at an ordinary lamp, and when in focus, the microscope is placed in the stand and firmly fixed in its place by the clamps. The lamp is then brought to its proper position, and the whole may be passed round. In about two minutes the specimen may be changed and another placed in its stead.

By this plan I have been able to show twelve preparations magnified from 15 to 500 diameters, to a class of upwards of a hundred during an hour's lecture. The condenser, mirror, diaphragm, may also be made to slide upon a rod fixed to the lower part of the stage as shown in Fig. 27, Plate X.

I have had an arrangement adapted to this microscope which enables me to use it for demonstrating structures with still higher powers. In the instruments used at my lectures given in 1861 at the College of Physicians, I was able to use successfully all powers up to the twelfth (700 diameters), and I feel quite satisfied that the plan will succeed equally with the highest powers which have ever been made. An instrument is now being made to take the  $\frac{1}{2}$ "<sub>11</sub>, the highest power yet made.

These hand microscopes can also be readily arranged in a line (Plate XII, Figs. 38, 39), or in a six or eight-sided frame (Figs. 36, 37), in the centre of which the light to illuminate all the objects at once may be placed.

One advantage of this arrangement for demonstrating to a class is that while every one can alter the focus to suit his vision the preparation and light are quite out of reach.

**22. Dissecting Microscope.** — The best form of dissecting microscope is that devised some years ago by the late Professor Quekett. Figs. 44, 45, 46, Plate XIV, show the instrument folded up, with an India-rubber band round it, in a manner which admits of its being carried in the pocket. Fig. 45 shows the internal arrangement and the manner in which the mirror, lenses, and lens-holders are packed away. The straight flat bar on the left serves to keep the legs from closing together (see Fig. 44), and also as a support for the mirror which slides into a piece of brass tubing attached to the flat bar. The instrument is furnished with three lenses, and is to be purchased at a moderate price.





Fig. 44.



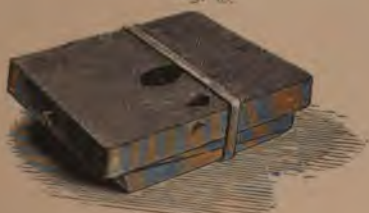
§ 22.

Fig. 45.



§ 22.

Fig. 46.



§ 22.

PROF. QUEKETT'S DISSECTING MICROSCOPE.

Arranged for observation.  
Mode of packing the different pieces of apparatus.  
The instrument folded up for travelling.



## CHAPTER II.

*Examination by Reflected Light, Transmitted Light, and Polarized Light.* REFLECTED LIGHT.—*Different Methods employed—Bull's-eye Condenser—Metallic Side Reflector—Lieberkuhn's—Dark-ground Illumination—Parabolic Illuminator—Annular Condenser.* TRANSMITTED LIGHT.—*Diaphragm—Achromatic Condenser—Gillett's Condenser.* POLARIZED LIGHT.—*Polarizer—Analyzer—Ice-land Spar—Tourmaline—Iodo-quinine or Herapathite.* ILLUMINATION BY ARTIFICIAL LIGHT.—*Lamps—Camphine Lamp—Oil Lamps—Argand Lamp—French Moderator Lamps—Gas Lamps—Of the Importance of Protecting the Eyes from the Diffused Light of Lamps.* ON DRAWING MICROSCOPICAL SPECIMENS.—*Camera Lucida Steel Disc—Neutral Tint Glass Reflector—Arranging the Light—Of making Drawings which it is intended should be Engraved—Tracing Paper—Retransfer Paper—Wood Blocks—Of obtaining Lithographs of Microscopical Drawings—Drawing on Transfer Paper—Drawing on the Stone—Of Engraving on Stone—Transfer Paper—Lithographic Ink—Lithographic Stones—On the Importance of Observers Delineating their own Work.* ON MEASURING OBJECTS.—*Cobweb Micrometer—Test Objects—Vohert's Lines—Jackson's Eye-piece Micrometer—Stage Micrometer—Simple Directions for Measuring Objects—On ascertaining the Magnifying Power of Object-glasses—To ascertain the Diameter of an Object—Standards of Measurement—Conversion of Foreign Standards of Measurement—On Finders—On Measuring the Angles of Crystals: Goniometers.*

## REFLECTED LIGHT, TRANSMITTED LIGHT, AND POLARIZED LIGHT.

FROM the most cursory examination of objects we learn that their internal structure differs materially in character from the external surface, and if the internal arrangement, as well as the external surface of an object, be examined by the microscope, the

observer will form an idea of its nature very different to that which he would have arrived at if he had regarded one set of characters only. Again, by employing polarized light we may often make out points in the structure of an object which cannot be perceived when it is examined by ordinary light.

I must therefore direct attention to the three following methods of directing the light upon objects submitted to microscopical examination.

1. *Reflected Light*, in which the light is thrown down upon the object, and the peculiarities of its surface alone observed, as in looking at different objects under ordinary circumstances.

2. *Transmitted Light*. The second mode of examination is by the aid of transmitted light, by which any inequalities in the internal structure of an object are demonstrated.

3. *Polarized Light*. By means of which the internal structure of various transparent objects may be rendered evident, in a manner in which they cannot be demonstrated by ordinary illumination.

*Reflected light* may be employed for the examination both of transparent and opaque objects, but *transmitted light* is only adapted for the examination of transparent structures. Every object to be examined by transmitted light should be very thin, or must be rendered transparent by some special method of preparation. To view an object by reflected light, the light must be thrown down upon it from above, by employing either the direct rays from a luminous body, or by the aid of a reflector; but in order to see the internal structure of a transparent object by transmitted light, the light must be so placed that the rays can pass directly through it, or they must be reflected upon its lower surface from a mirror placed beneath it, and arranged at the proper angle.

33. *Reflected Light*.—The light employed may be ordinary *daylight*, *sunlight*, or the *light* of a *candle*, or *good lamp*. The most important modes of illuminating objects for examination by *reflected light* are the following:—

34. *Daylight*.—1. By ordinary *diffused* daylight, *sunlight*, or *lamp-light*; but *diffused light* will usually be found insufficient for *good illumination*.

Fig. 46.

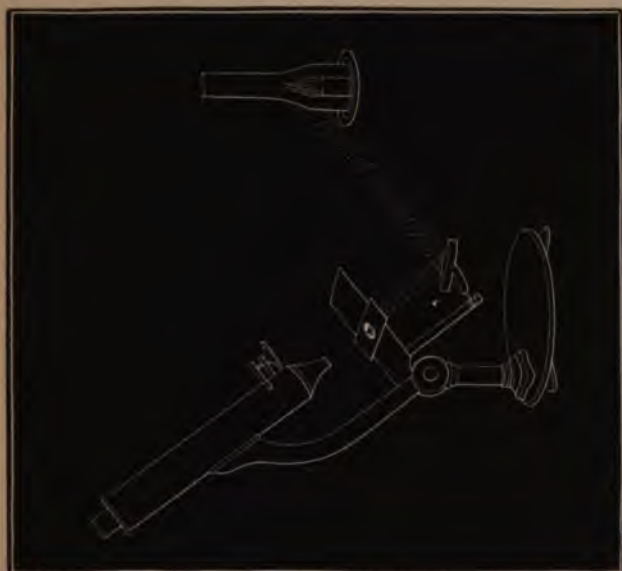


Fig. 48. Diagram to show the arrangement for examining objects by transmitted light (§ 30).

Fig. 47.

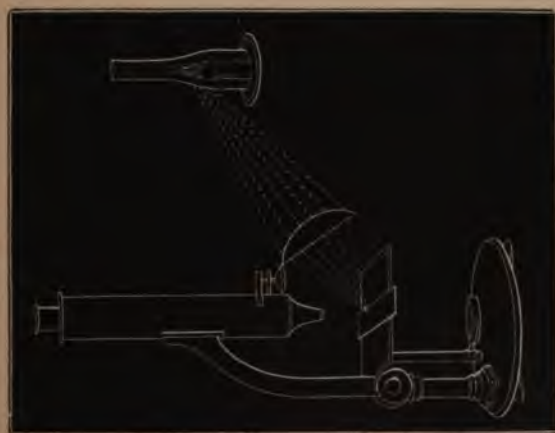


Fig. 47. Diagram to illustrate the arrangement for examining objects by reflected light (§ 25).





25. **Bull's-eye Condenser**.—2. By condensing the light upon the object by means of a large simple plano-convex lens, or *bull's-eye condenser* (Figs. 49, 50, Plate XVI), or when very high powers are required, by a combination of two lenses, conveniently arranged. The ordinary arrangement for examining objects by reflected light is represented in Plate XV, Fig. 47.

26. **Metallic Reflector**.—3. By causing the light to be brought to a focus upon the object by means of a small concave *metallic reflector* fitted upon one side of the instrument.

27. **Lieburkuhn**.—4. By causing the rays of light reflected from the mirror and passing round the *circumference of the object* to impinge upon a *concave annular reflector* or *Lieburkuhn* adapted to the object-glass, from which the rays are reflected downwards, and brought to a focus upon the surface of the object itself (Plate XVII, Fig. 56). The light is prevented from passing directly through the object by a small metal stop, *e*, or even by a piece of black paper placed immediately beneath it, and corresponding to the aperture of the object-glass. This arrangement will be readily understood by referring to the figure.

The *first* mode of illumination seldom affords sufficient light to show the character of the surface satisfactorily. The *second* and *third* plans are those usually adopted, and are the most convenient as well as the most efficient modes of illuminating the surface of objects. The *fourth* method is now seldom resorted to, and is only applicable in cases where the object is small enough to permit the passage of a sufficient quantity of light around it. If a transparent object is to be examined by reflected light, a piece of black paper, rather larger than the aperture of the object-glass, should be placed behind it to prevent the passage of light through it, or one of the stops supplied with some instruments may be inserted in its place beneath the stage. The stops, however, are not furnished with many of the modern microscopes, as the second and third modes of illumination, and those next to be described, afford the most satisfactory results.

28. **Dark-ground Illumination**.—In this place I must allude cursorily to a mode of illumination which has been much in repute of late years, and which is very advantageous for demonstrating some structures. I refer to *dark-ground illumination*, in

which the object appears in relief upon a black ground. This mode of illumination is particularly applicable to investigations upon some very minute organisms, such as the diatomaceæ. The appearance produced is very different to that obtained by merely throwing the light upon the surface of the object, and many points may be learned with reference to the nature of the markings upon a specimen which could not be ascertained by the ordinary methods of directing the light upon it. In this mode of illumination the direct rays are prevented from penetrating the specimen, and passing through the object-glass, but the preparation is highly illuminated upon all sides by light made to impinge upon it in a very oblique direction. Thus the object is thoroughly illuminated at all points, but the ground on which it lies, appears perfectly dark. There are several methods by which this result may be obtained. One very simple little instrument is termed a *spot-glass*,\* and consists of a plano-convex lens, the convexity being so great that rays of light passing through it would be made to converge with a great degree of obliquity, and would be brought to a focus at a short distance above the flat surface of the lens. In the centre of the flat surface is placed a small circular piece of black paper in order to prevent the passage of any direct rays of light. The lens is fixed in a brass tube made to slide up and down, so that it may be adjusted at the proper distance below the object.

29. The *Parabolic Reflector* of Mr. Wenham, Mr. Shadbolt's *annular condenser*, and the *parabolic illuminator* of Messrs. Smith and Beck are beautiful instruments for effecting the same purpose in a more efficient manner (*see* Plate XVII, Fig. 58). Another excellent plan has lately been devised by Mr. Wenham, the simplicity of which recommends it strongly to our attention. A small triangular prism is placed beneath the object, so that one of its plane surfaces is in contact with the under surface of the slide carrying the object. The light is refracted so highly that none passes directly through the object, but, being thrown at the proper angle upon the under surface of the thin glass which covers it, is entirely reflected from thence upon the object itself, which is thus highly illuminated.

The Rev. J. B. Reade has contrived a very useful hemispherical

\* The spot-glass may be obtained of the different microscope makers for about 7s. 6d.

Fig. 49.



§ 25.

Fig. 50.



§ 25.

Fig. 55.

Fig. 51.



§ 44.

Fig. 52.



§ 43.

Fig. 53.



§ 35.

Fig. 54.



§ 39.



Fig. 49. Bull's eye condenser, for student's microscope.

Fig. 50. Bull's eye condenser, on upright stand.

Fig. 51. Neutral tint glass reflector, for drawing.

Fig. 52. Steel disk.

Fig. 53. "Polarizer," placed beneath the object.

Fig. 54. "Analyzer," placed above the object.

Fig. 55. Lamp (Messrs. Smith & Beck), for camphine or belmontine.





condenser for examining objects exhibiting very delicate lines by oblique light (*see* Trans. Mic. Society, 1861, p. 59).

In employing the spot-glass or parabolic illuminator the light should be reflected from the plane mirror.

**30. Transmitted Light.**—In discussing the mode of illuminating objects by transmitted light, I must briefly draw attention to two or three beautiful instruments for condensing the light upon the object. The microscope, in Plate XV, Fig. 48, is arranged in the ordinary position for examining transparent objects. The light may be received upon the plane or concave mirror, according as a moderate or brilliant light is required; but, as a general rule, the intensity of light should not be greater than necessary to make out distinctly the structure of the object. Direct sunlight is not to be employed, and a very strong light of any kind is hurtful to the eyes. The best light during the day is to be obtained from a white cloud upon which the sun is shining.

**31. Diaphragm.**—Beneath the stage in most microscopes is placed a plate with holes in it of different sizes. This is the *diaphragm*, and is employed for cutting off the most oblique rays and superfluous light. Every microscope should be provided with a diaphragm, fitted on about an inch beneath the stage, and arranged on a pivot, so that any of the holes may be brought under the object (Fig. 9, Plate II, §12). The definition of the structure of a transparent object is often found to be very much clearer when only the more direct and central rays of light from the concave mirror are allowed to pass through it.

**32. Achromatic Condenser.**—The illumination of some objects examined with high powers is much improved by causing the light to pass through an achromatic condenser which consists of an ordinary achromatic objective of half or a quarter of an inch focus, arranged in a sliding tube immediately beneath the stage. One of these instruments can be fitted to the student's microscope. Mr. Quekett has adapted a simple lever handle by means of which the right focus is readily obtained (Plate XVII, Fig. 59). The instrument is not an expensive one if it be made of a French combination. Practically, however, I have obtained very good illumination for the examination of tissues without using an achromatic condenser. Indeed, I have found in many cases that

I could see more clearly without it than with it. A Kelner's eye-piece, however, as already stated, makes a most valuable achromatic condenser, and is of the most material assistance in using very high powers. The lenses used for condensers are usually much too small and the power too high.

**33. Gillett's Condenser.**—Mr. Gillett has adapted a diaphragm plate and stops to the achromatic condenser, and there is a beautiful instrument of this kind made by Mr. Ross. Messrs. Powell and Lealand have, however, improved upon it, and brought out a much smaller and more compact condenser, which is attached to their microscope.

**34. Polarized Light.**—In examining an object by polarized light it is necessary to have one crystal beneath the object, termed the *polarizer* (Plate XVI, Fig. 53), and fitted under the stage, and another one above the stage, inserted into the tube above the object-glass, or adapted to the eye-piece—this is termed the *analyzer* (Fig. 54).

**35. Iceland Spar.**—Various crystalline substances are employed for polarizing the light. Two crystals of *Iceland spar* are usually preferred. These are divided obliquely, and connected together again with Canada balsam so that one of the two images formed by this double refracting crystal is removed from the field of view (Plate XX, Fig. 69). *Tourmaline* crystals have also been used, but their colour is a disadvantage.

**36. Iodo-quinine.**—By the kindness of Dr. Herapath I have received two beautiful crystals of the *Iodo-quinine*, or *Herapathite*, prepared by him for polarizing the light. These are mounted between two pieces of thin glass, and they effect the object for which they are intended perfectly well.

**37. Objects Examined.**—It is most instructive for the observer to subject specimens of the same object to examination in four different ways. He will not fail to notice the very different appearance presented by the object. 1. The surface of the object may be examined by *reflected light* brought to a focus upon it by means of a bull's-eye condenser. 2. The light may be *reflected* upon it from a *Lieberkuhn*. 3. The light may be transmitted.

Fig. 56.



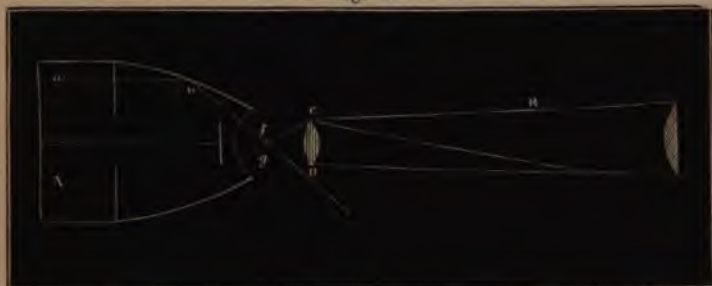
§ 27.

Fig. 57.



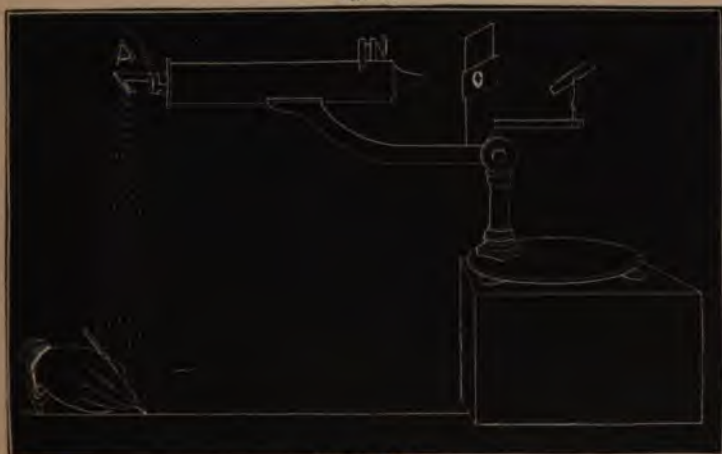
§ 32.

Fig. 58.



§ 29.

Fig. 59.

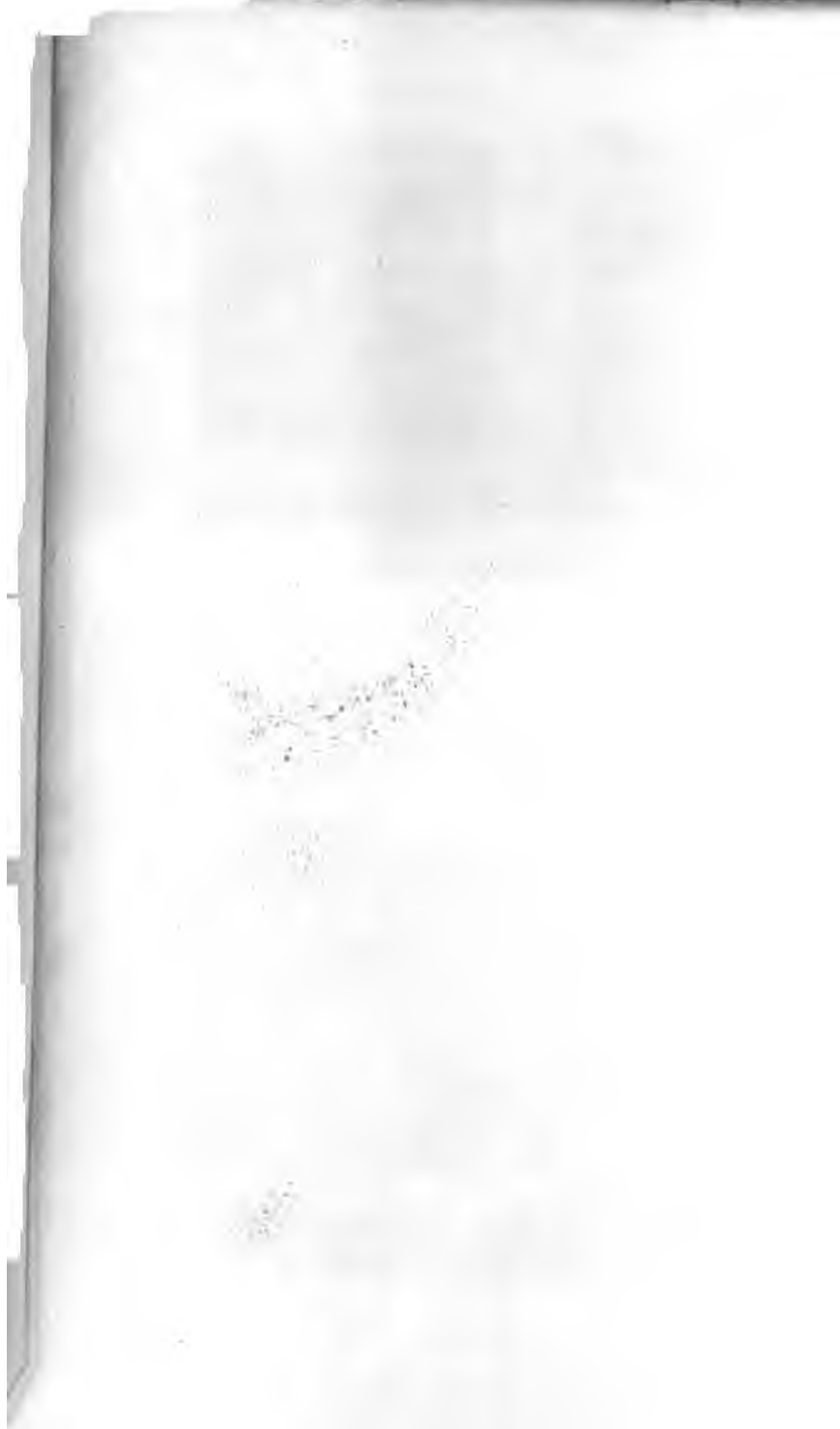


§§ 41, 44, 45, 62.

Fig. 56. To illustrate the mode of examining an object by reflected light with the Lieberkuhn. The light reflected from the mirror, *a*, passes through the glass slide, *b*, around the object, and impinges on the concave annular mirror, *d*, by which the rays are brought to a focus and condensed upon the object placed at *c*. Fig. 57. Achromatic condenser mounted with a lever handle. Fig. 58. Parabolic illuminator, showing course of a ray of light, *m, n, f*, when an uncovered object, *g*, is placed in focus. Fig. 59. Position of microscope for drawing and measuring objects.

[To face page 24.]





through the object after it has been reflected from the surface of the mirror. And, 4. The object may be placed under the influence of *polarized light*.

Now the very different appearances observed should be carefully noted, and after each examination the student should endeavour to form a notion of the probable nature of the substance, and the precise arrangement of the elementary parts of which it is made up. Lastly, the conclusion arrived at with reference to the nature of the structure after having been submitted to these four modes of examination should be contrasted with the idea which would have been formed of it if an observation had been made by one mode of illumination only.

**38. Artificial Illumination.**—It may be said with truth that microscopical work should, if possible, be undertaken only by daylight, since the most perfect artificial light which can be obtained is far inferior for delicate observation, while it strains the eyes very much more. Still many of us are compelled by necessity to work principally by night, and it is therefore a matter of the greatest importance to be provided with the best kind of artificial illumination.

**39. Lamps.**—From time to time various microscope lamps have been proposed. The small *camphine lamp* of Messrs. Smith and Beck represented in Plate XVI, Fig. 55, is one of the most perfect small lamps which I have seen. It gives a beautiful white light, and produces very little heat. Of *oil lamps* there are several which serve for microscopical examination. The *German Argand lamp*, lately imported into this country by Mr. Pillischer, is a good microscope lamp, and so also is the *French moderator*, especially if provided with a blue or neutral tint glass, and a shade. The common paraffin lamps give a most excellent light for the microscope, but Belmontine affords still better and whiter illumination. A cheap and at the same time very efficient microscope lamp is still a desideratum.

**40. Gas Lamps.**—For those provided with gas I recommend very strongly the gas lamp of Mr. Highley, which is provided with a flat brass plate and a water bath, instruments of great use in microscopical investigation. The light is made to pass through an opening in a diaphragm, so that the eyes are quite protected from

the diffused light. A very pleasant light is produced by causing the rays to be transmitted through a blue chimney glass and a flat piece of neutral tint glass. The only objection to this lamp is its great heating power. One of these lamps is represented in Plate XVIII, Fig. 66.

In all cases the eye should be carefully protected from the dazzling light when not employed in looking through the instrument. The eye not observing should always be kept open, but should be protected from the direct glare of the microscope-lamp. For this purpose a shade made of black paper may be fitted to the body of the instrument at a convenient distance below the eyepiece. In all cases the light should be perfectly steady, and so situated that it may be conveniently reflected upon the object by the mirror.

#### OF DRAWING, ENGRAVING, AND MEASURING OBJECTS.

41. *Of Drawing Objects.*—It may almost be said that all real advance in our knowledge of the minute structure of both animal and vegetable tissues, depends upon the drawings which are made. It is almost hopeless for an observer to attempt to describe what he sees in words, and such descriptions, however careful they may be, cannot possibly be compared with those of others. On the other hand, a truthful drawing of what a man has seen lately may be compared with others which may be made a hundred years hence, although the means of observation will be far more perfect then than they are at present. Much may be learned by such comparisons. I am sure that an honest inquirer cannot be of greater use in his time than by making good drawings of what he has seen;—they will be of far greater help to our successors than any amount of description we can write for them, and we may feel sure they will look at our drawings if they are honest copies of nature, while we all know that comparatively very little of what we write will be read when the whole aspect of this department of science shall be changed, as it will be.

In delineating an object magnified by the microscope it is important to copy it correctly, both as regards the relative position of the several structures to each other, and also with respect to size. To copy the size exactly will be found extremely difficult by the eye alone, but there are several ways of proceeding by

which accuracy may be ensured. Some of these I shall now briefly describe. The simplest method is to place the paper upon the same level as the stage upon which the object is situated. If we now look steadily at the object with one eye, while the other is employed to govern the movements of the pencil, the object will appear to be thrown, as it were, upon the paper, and its outline may be very readily traced. By a little practice the relative size of objects may be insured in this manner, but it is troublesome and difficult to keep both the object and paper perfectly still.

**42. Camera Lucida.**—The principle of the camera lucida has been applied to taking microscopical drawings, and has been found to succeed admirably. The object appears to be thrown down upon the paper, and with a little practice the observer may trace the lines with great accuracy.

**43. Steel Disk.**—If a little steel disk be placed at an angle of  $45^{\circ}$  with the eye-glass, it will receive the magnified image of the object and reflect it upwards upon the retina of the observer. The disk is smaller than the aperture of the pupil, and the pencil can at the same time be seen very well as it traces the image apparently thrown down upon the paper beneath. The steel disk is represented in Plate XVI, Fig. 52.

**44. Neutral Tint Glass Reflector.**—The simplest and cheapest reflector for microscopical drawing, consists of a small piece of plate-glass slightly coloured, in order to improve its reflecting power, but still not so dark as to prevent an object being seen through it perfectly. This is also arranged at an angle of  $45^{\circ}$  with the eye-glass, and the draughtsman can very easily follow his pencil upon the paper. This instrument is represented in Plate XVI, Fig. 51.

In order to use these instruments, the microscope is arranged horizontally, and the paper placed on the table, as shown in Plate XVII, Fig. 59.

**45. Arranging Light.**—It is important, however, in using these instruments, to arrange the light carefully. The image should not be illuminated too intensely, and the paper upon which the drawing is made should not be too much in the shade, or the



point of the pencil will not be seen distinctly. Experiment can alone decide the relative intensity of the light upon the object and upon the paper, but with a little practice the proper amount of illumination will be discovered. The distance between the reflector and the paper should be precisely the same as from the object to the eye-piece, for otherwise the size of the object delineated will be altered.

The object appears to be thrown upon the paper, and its outline is very readily traced. If it is to be drawn smaller, it is only necessary to place the paper upon a stand closer to the reflector. If, on the other hand, a large *diagram* is required, the distance must be increased. By placing the diagram paper upon the floor, the object can be readily traced with a long pencil. In this manner many of my diagrams have been made. They must of course be accurate copies of the objects themselves, and are therefore far more truthful than diagrams copied from drawings representing microscopical structure, can be. If the distance of the diagram paper be always the same, the drawings so obtained may be compared with each other, and scales of measurement may be appended to them by proceeding in the manner described in

**46. Of making Drawings which it is intended should be Engraved.**—With a little practice, the observer may acquire the power of drawing on wood, and the engraver will often be able to produce a more faithful representation of the object than he could by copying the drawings of the microscopical observer. It is, however, necessary to practise the plan of producing varieties of tints, by straight lines, whenever this can be done, as the labour of engraving is thus much economised. The drawing should first be made roughly on paper, in order to obtain the size and general characters of the object. A piece of retransfer paper is then placed upon the prepared block, and the prominent lines of the drawing retraced with some blunt-pointed instrument (a needle, the point of which has been made blunt by filing it, answers very well). By using a slight pressure, the colour of the retransfer paper is transferred to the wood block in the lines corresponding to those of the drawing. These lines are afterwards reproduced by lead pencil, corrected, if necessary, and the delicate parts of the drawing filled in by carefully copying from the object.

If the engraving is to be a fac-simile of the drawing with the different parts on corresponding sides, it is necessary, in the first place, to copy the picture with ordinary tracing paper, and *invert* the tracing upon the retransfer paper on the wood block, as the impressions are of course always reversed; or a reverse may be obtained by copying the image of the drawing reflected from a looking-glass. Beautiful specimens of wood engraving are seen in some of the plates in Chapters V, VI and X.

47. **Tracing Paper** is a very transparent paper, obtained by soaking tissue paper in some oily material, and allowing it to dry.

48. **Retracing Paper** consists of tracing paper, upon one side of which a fine red or black powder has been rubbed, which adheres to the paper pretty firmly, but which, at the same time, may be made to adhere to another surface by firm pressure.

49. **Wood Blocks** are *prepared* by rubbing a little dry carbonate of lead and brick dust moistened with water upon the surface, and allowing a very little to dry on. In this way a smooth white surface is obtained, admirably adapted for receiving the most delicate drawing. It is well to moisten the white lead with a little very weak gum water, which makes it adhere firmly to the surface and gives a very smooth face. Wood blocks may be obtained of Williamson and Son, Picket-place, Strand. Every observer should draw on the wood block himself.

50. **Of obtaining Lithographs of Microscopical Drawings.**—I think it desirable to give a few directions for drawing on stone, as I believe there are many observers who would willingly give up the necessary time required to place their work on the stone, who could not afford to employ a lithographic artist. I made many drawings in this manner some years ago, and with the help of a boy, who could at first draw but little, have been able to publish numerous drawings, which are very accurate copies of the objects, although in execution will not bear comparison with artists' work.\*

51. **Drawing on Transfer Paper.**—If the drawing does not contain much very minute work, it may be drawn on properly

\* See the earlier numbers of my 'Archives.'

prepared transfer paper with lead pencil, direct from the microscope. Afterwards, the lines are to be traced with a pen with lithographic ink; the shading may be effected by delicate lines made with the pen, or with lithographic chalk. The latter plan, however, is not well adapted for making transfer drawings. The drawing is then to be sent to the lithographic printer, where it is damped, placed downwards on a dry stone, and after being subjected to firm pressure, the paper may be peeled off, leaving the preparation, with the drawing, on the stone. The latter is removed with water, the drawing properly set, and then the printing ink applied with the roller.

**52. Transfer Paper** is prepared for the purpose. That which was made of India paper, I found answered exceedingly well.\*

**53. Drawing on the Stone.**—There are two plans for drawing on the stone itself, which produce better results than the preceding method, but they require more practice for their performance. When much shading is required, and extreme delicacy of outline is unnecessary, the outline is first made on paper, and the drawing retraced on the stone in the manner designated in § 46; the outline may then be traced with ink, a pen, or very fine sable hair brush, being used for the purpose; the shading is to be given with the lithographic chalk. The chalk is to be very finely pointed by cutting downwards, the point being uppermost (as in pointing an ordinary chalk crayon), and held in a handle made out of a common quill. The lines are to be made very gently, repeating the strokes frequently with a light hand, when depth of colour is required, rather than by leaning heavily so as to remove a considerable quantity of chalk at once, and deposit it upon the stone. When chalk shading is employed, a finely *grained* stone is required.

**54. Of Engraving on Stone.**—If the work is very delicate, as is the case with most subjects the microscopical observer wishes to obtain representations of, engraving on stone will give the most satisfactory results. The process is very simple, but requires considerable practice in executing it. The stone must be finely polished, and it is well to have it tinted with a little infusion of

\* To be obtained of Messrs. Harrison and Sons, St. Martin's Lane.



logwood, or to cover it with a thin layer of lamp black, which enables the draughtsman to see his strokes better. The outline of the drawing is traced as before, and then the lines scratched upon the stone with a very fine point. A needle point, previously hardened by being heated red hot and suddenly dipped in cold water, inserted into a strong handle, may be used. I generally use an etching needle; the point requires to be sharpened from time to time upon a hone. But a properly made diamond point is far better. The dark parts are shaded by lines placed very close together, or cross shading may be adopted, or the tint may be given by dots, as in copper-plate engravings. Generally it is better to try to obtain the appearance of texture by copying, as nearly as possible, the character of the tints of the object itself. The thickness of the line in the print will depend upon the width of the line, without reference to the *depth* to which it extends into the stone. It is desirable to make two or three narrow lines near to each other, instead of one wide one, when a thick line is required. After all the lines have been scratched, the stone is sent to the lithographic printer, who will obtain impressions from it. The oily material only adheres to the rough scratches, and subsequently when the stone is wetted, the ink only attaches itself to the oily parts.

**55. Lithographic Ink.**—The ink may be obtained in the fluid state, but it is better to use the solid ink, a little of which is rubbed up with water when required.\*

Lithographic chalk may be obtained of different degrees of hardness,—it can always be made much harder by melting it and rolling it into sticks.

**56. Lithographic Stones.**—The stones are sold by the pound. It is desirable to obtain stones large enough to hold four octavo pages of drawings, as the expense of working a stone of this size is little more than that only large enough to contain one.

\* The apparatus, ink, chalk, &c., alluded to, can be obtained of Messrs. Waterlow, Messrs. Hughes and Kimber, Red Lion Court, Fleet Street, and most lithographers. It is only due to Messrs. Harrison, of St. Martin's Lane, that I should thank them for the kindness they have always displayed in assisting me in carrying out this and many other plans of producing drawings. Without the important help they and their workmen have afforded me on all occasions, my efforts would probably have failed, as I had no knowledge of practical lithography.

57. On the importance of observers delineating their own work.—It will, I know, be said that these processes take much time, and after all are of a nature which an intelligent draughtsman can perform, and hardly worth the labour which a microscopical observer, who wishes to carry them out, must be content to bestow. Objections of other kinds may be urged, but I cannot but feel that if I had been prevented from having the drawings made at home, not one of the pages illustrating many of my works would have been published. I remember how much I needed at one time the little information given here—and I therefore gladly communicate it, imperfect as it is, in case there may be others in the same situation as myself. Now, I believe that it is quite as impossible to obtain a good representation of any microscopic object without long and careful study, as it is to produce a copy of any other object in nature; and surely it is hard to expect a draughtsman, who is engaged in copying various subjects, to spend hours in looking at specimens in a microscope, observing things which he neither knows nor perhaps desires to know anything about. Neither is it possible that any one man can make himself fully conversant with all the beautiful minutiae in every branch of microscopic inquiry. It is true that Mr. Tuffen West, and one or two other gentlemen, have taken up this kind of drawing and engraving, and have produced most beautiful results. I believe Mr. West's success as an engraver of microscopic objects to be due to the interest he takes in the subject, and to his being himself a practical microscopical observer.

There are many drawings of microscopic objects which ought to be published, and although these may be of little interest to persons generally, are absolutely required by those who are working at special subjects. Now, however rich a man may be, it is doubtful if a large sum of money should be spent in employing artists to do work which, however well skilled, they cannot do so truthfully as the observer himself, unless they had devoted the same attention to the subject. Few have time or inclination to do this. There is not the same question about our own time. Whatever is worth doing at all, and is worth recording, is worth an expenditure of time, is worth doing well—though it may involve some sacrifice on our part. Whatever is observed is worth copying, provided it has not been correctly copied before. It would, I think, be quite possible for

many to learn the process of drawing on stone, and thus engrave many drawings of great use which would not otherwise be published.

Very much yet remains to be done in representing microscopic texture faithfully. Photography has done much, and will, doubtless, assist more, but there are many structures the colour of which alone renders it quite impossible to obtain photographs of them. It can only be by patient study, that any one can hope to be able to copy accurately by hand the beautiful and delicate lines and tints in many microscopic objects, but it is so important that this should be done well, that I cannot too strongly urge on all those who wish to work at the microscope, earnestly to practise drawing as much as possible.

All advance in our knowledge of structure, as well as of the minute changes incessantly going on in living organisms, depends I think, in great measure, upon accurate copies of the objects being made, for in this way alone can the work of the present generation be useful to that which succeeds it.

It is beyond the power of language to describe the characters of many structures in such a way that their appearance could be reproduced in the mind of another, and even if this could be done, so wonderfully delicate and minute are the observed differences in many cases, that any attempt to classify and arrange our observations seems at present hopeless; and becomes more hopeless in proportion as observations multiply; while the different meaning which different persons attach to words and phrases, introduces another difficulty in our attempt to collate and deduce inferences from the observations which have been made.

Take for instance morbid structures. Although we possess observations without end, we are not yet able to group them under general heads, nor are we acquainted with their mode of origin, or conversant with the changes which take place in their anatomical elements at different periods of their growth. Much difficulty has arisen from the attempts to assign definite names to various growths, while the precise characters included under any particular term have never been properly defined—and it is doubtful if this can be done except by a nomenclature, the complex nature of which would be fatal to its introduction. Now surely our knowledge on these and other subjects would have been much more extensive and more accurate, if instead of



long descriptions we had been furnished with sketches of the morbid growth, its dimensions and weight, the length of time it had been growing, and a few general points in the history of the case, with accurate drawings of the minute structure of the tumour. It is true that all persons cannot draw well, but a very little patience will enable any one to copy a microscopical specimen, and an accurate copy, although it be very badly executed, has an aspect of truth which is unmistakeable, while a drawing which is the offspring of the imagination instead of a simple copy of nature, bears the mark of untruth in every line, however elaborate and unexceptionable its execution may be. Errors of observation are, I believe, much more easily detected in a drawing than in verbal description. A mistake or misinterpretation expressed in a drawing can, and at length must be, corrected by subsequent observation, while ill-observed or misinterpreted facts, cloaked in obscure language, may be propagated for years, and no matter how false they are, it may be very difficult to refute them. I would, therefore, urge upon every one the importance of making drawings at whatever cost of time and labour; it is worth any sacrifice to do really good work, and if every observer could but record a few accurate observations during his life, the united labour would indeed be productive of great results.

I would also strongly urge upon observers the importance of at once agreeing upon some general plan of delineating objects, so that our observations may be useful to all, while the task of those who will hereafter have to arrange and deduce conclusions from our work will be much facilitated. The value of many beautiful drawings would be greatly increased if a scale of 100ths or 1000ths of an inch was appended to them, and the magnifying power of the object glass stated. This would not have added five minutes to the time required for the task, while it would have rendered the drawings comparable with others. In some, the magnifying power is not even mentioned, and in others there is reason to believe it is wrongly stated.

Every one who copies an object should state the magnifying power of the combination of lenses he employed, and should append a scale magnified by the same combination (*See* § 62).

Let it not be supposed that I am insensible to my own shortcomings in these and many other matters. I am conscious that every drawing I have published might have been, and ought to

have been—better,—and I can only hope that the desire for seeing our work useful to each other and to our successors, as well as to ourselves, will be received as a sufficient apology for these remarks.

#### ON MEASURING OBJECTS.

Most of the larger and complete microscopes are furnished with micrometers adapted to the instrument, but it appears to me that the simple method of measuring objects, presently to be described, to a great extent supersedes the necessity of those more expensive arrangements. It will be well for me, perhaps, in the first place, to describe briefly the different forms of micrometers in use.

**58. The Cobweb Micrometer**, originally applied to telescopes by Ramsden, its inventor, is a beautiful instrument, which can be fitted to the upper part of the body of the microscope. A fixed cobweb crosses the field of view, and parallel to this is another cobweb thread capable of being brought near to, or separated from the first, by turning a milled head, to which is attached a graduated circle. The value of each degree on the circle is ascertained by placing an object of known dimensions, as the *stage micrometer* graduated to thousandths under the object-glass, and ascertaining the number of degrees on the screw which correspond to the 1-1000th of an inch. From these data a simple table may be constructed, and the diameter of any object can be readily ascertained by bringing one side of it up to the fixed line, and causing the moveable line to touch the opposite. If we ascertain the value of the degrees as marked upon the circle when the lines are separated at the proper distance, we may estimate directly the diameter of the object. The older observers used to measure objects by means of very delicate wires, separated from each other by certain known distances placed in the focus of the eye-piece, or by employing points, one of which could be moved from or towards the other by means of a screw.

**59. Jackson's Eye-piece Micrometers.**—Mr. Jackson arranged a micrometer slide in the eye-piece so that it could be brought over the magnified image of the object by means of a screw.

**60. Stage Micrometers.**—Within the last few years, lines, separated from each other by certain known but very minute intervals, have been ruled upon slips of glass by means of a diamond attached to a beautiful instrument, provided with a most delicate arrangement for moving it the required distance from the last line engraved. A second line is then ruled, then a third, and so on. Excellent stage micrometers of this kind have been ruled by Mr. Jackson. They can be obtained of all the instrument makers; but they are now made by Messrs. Powell and Lealand.

**61. Test Objects.**—To such wonderful perfection has this process been carried, that M. Nobert, of Griefswald, in Prussia, has engraved lines upon glass so close together that upwards of 80,000 would go in the space of an English inch. Several series of these lines were engraved upon one slip of glass. By these the defining power of any object-glass could be ascertained. As test objects, they are equal to, and even rival, many natural objects which have hitherto been employed for this purpose. The delicate lines on some of the diatomaceæ are separated from each other by the 1-50,000th of an inch, while the finest lines engraved by M. Nobert are not more than the 1-100,000th of an inch apart.

In order to measure the diameter of an object the glass slide upon which the lines have been engraved (1-1000th or 1-100th of an inch apart according to the magnifying power) may be placed beneath the object upon the stage. This arrangement, however, is only suitable for low powers, since the object and lines cannot be in focus at the same moment, so that it is impossible to obtain a very correct measurement.

The podura scale is a most excellent "test object." According to Prof. Bailey of the United States, *Grammatophora subtilissima* and *Hyalodiscus subtilis*, are the most delicate tests ("*Smithsonian Contributions*," Vols. II and VII; also a paper by Mr. Hendry, "*Quart. Journ. Mic. Science*," Vol. I, p. 179, 1861; one by Messrs. Sullivant and Wormley, "*Silliman's American Journal*," Jan. 1861).

For testing the penetrating power of an object-glass, very fine nerve fibres, as, for example, those distributed to vessels, or very delicate fibres of striated muscle, mounted in glycerine, may be employed. It should be borne in mind that the object glasses with a very high angle although very valuable for researches



upon the diatomaceæ, and other very delicate objects of extreme tenuity, do not answer so well for investigations upon "the structure of animal and vegetable tissues, as glasses of a moderate or very low angle. This question is fully discussed in the remarks on "*Test Objects*," by Dr. Carpenter, "*The Microscope and its Revelations*," pp. 141, *et seq.*

**62. Simple Method of Measuring Objects.**—The most simple and efficacious manner of measuring objects is with the aid of the camera or neutral tint glass reflector referred to before (§ 44). In the field of the microscope is placed an ordinary micrometer, with the lines separated by thousandths of an inch. Care being taken that the instrument is arranged at the proper distance from the paper, the lines magnified by a quarter of an inch object glass are carefully traced. The micrometer is removed and replaced by the object whose diameter is to be ascertained. In Plate XVIII, Fig. 60, both micrometer lines and objects are shown magnified by the same power. The object is traced over the lines, or upon another piece of paper, and compared with the scale by the aid of compasses. The lines may be engraved upon a slate, and their value affixed, so that any object may be at once measured. We require of course a different scale for each power. Such scales may be made upon pieces of gummed paper, and one of them may be affixed to every microscopical drawing. Fig. 61 shows several such scales magnified by different powers. Thus the size of every object delineated may be at once ascertained, and the trouble of making individual measurements saved, while at the same time the inconvenience of a long description of the dimensions of various objects is avoided, than which nothing can be more tedious or less profitable to the reader.

In comparing the representation of the same object delineated by different observers, it will be often found that great confusion has been produced in consequence of the magnifying power of the object-glass not having been accurately ascertained, and an object said to be magnified the same number of times by two authorities, is not unfrequently represented much larger by one than by the other. This discrepancy in most cases arises from the magnifying power of the glasses not having been accurately ascertained in the first instance.

I cannot too strongly recommend all microscopic observers to ascertain for themselves *the magnifying power of every object-glass*

and to prepare, in the manner presently to be described, a *scale of measurement by which the dimensions of every object can be at once ascertained.*

The plan of appending to every microscopical drawing a scale magnified in the same degree as the object represented, supersedes the necessity of giving measurements in the text, while it is free from any of the objections above referred to. With very little trouble, every one can prepare scales for himself.

**63. On Ascertaining the Magnifying Power of Object-glasses.**

—I will now describe the method of ascertaining the magnifying power of the different lenses. Although the several object-glasses are termed one inch, one quarter of an inch, one eighth, &c., the magnifying power of each is not definite, and the quarters of some makers magnify many times more than those of others. It is well, therefore, that every observer should be able to ascertain for himself the magnifying power of his different glasses. Suppose I wish to know how much a French quarter magnifies. The one-thousandth of an inch micrometer is placed in the field, and the magnified image is thrown by means of the neutral tint glass reflector upon a scale, divided into inches and tenths of inches. The magnified one-thousandth of an inch covers about two-tenths of an inch, and consequently the glass magnifies about 200 diameters; for if it covered one inch, the thousandth of an inch must have been magnified 1,000 times, but in this case it only corresponds to the one-fifth of an inch, and therefore the one-thousandth is magnified 200 times. For lower powers the one-thousandth of an inch scale may be employed. The manner of ascertaining the magnifying power is therefore exceedingly simple; but it is very important for the observer to know the magnifying power of every lens, and he should ascertain this before he commences to make any observations. This simple process will be readily understood if Fig. 62 in Plate XVIII be carefully studied. To carry out this plan it is only necessary to be provided with a stage micrometer divided to 100ths and 1,000ths of an inch, and an inch scale divided to tenths.

**64. To Ascertain the Diameter of an Object.**—If an object be substituted for the micrometer, and its outline carefully traced upon paper, its dimensions may of course be easily ascertained by

Fig. 60.



Fig. 61.



§ 63.

Fig. 63.

11	12	13	14	15
24	24	24	21	24
11	12	13	14	15
25	25	25	25	25
11	12	13	14	15
26	26	26	26	26
11	12	13	14	15
27	27	27	27	27

§ 67.

Fig. 65.



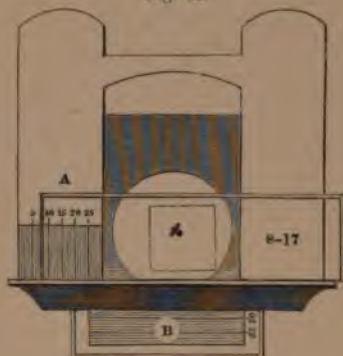
§ 67.

Fig. 61.

1000 lbs	.....	× 403
1000 lbs	.....	× 213
1000 lbs	.....	× 330
100 lbs	.....	× 33
100 lbs	.....	× 20
100 lbs	.....	× 25

§ 63.

Fig. 64.



§ 67.

Fig. 65.



§ 40.

Fig. 60. Lines separated by  $\frac{1}{1000}$  of an inch magnified 215 diameters, with objects magnified in the same degree. Fig. 61. Scales, hundredths and thousandths magnified in different degrees. Fig. 62. Mode of ascertaining the magnifying power of an object-glass. *a*. 1000ths of an inch  $\times 200$ . *b*. Each scale divided into tenths. *c*. 1000ths of an inch  $\times 130$ . *d*. 100ths of an inch  $\times 40$ . Each magnified 1000th of an inch covers two-tenths, or one-fifth of an inch, therefore the glass magnifies 200 times, for  $\frac{1}{1000} \times 200 = \frac{2}{10}$  or  $\frac{1}{5}$  of an inch. Each 100th of an inch covers four-tenths of an inch, therefore the glass magnifies 40 times, for  $\frac{1}{100} \times 40 = \frac{4}{10}$  or  $\frac{2}{5}$ . Fig. 63. A portion of Maltwood's finder, as seen in the microscope. Fig. 64. Simple finder, designed by Mr. Wright. Fig. 65. Instrument for scratching a circular line on the thin glass, to show the position of an object. Fig. 66. Gas microscope lamp, with water bath, &c., arranged by Mr. Highley. [To face page 35.]





comparison with the micrometer lines. The magnifying power used being the same in both cases.

In order to apply this plan to microscopical drawings generally, the following seems to be the simplest method of proceeding, and saves much trouble. Scales are carefully drawn upon gummed paper; the magnifying power, and the micrometer employed being written against them as represented in Plate XVIII, Fig. 61. If a number are drawn together, one of the rows can be cut off and appended to the paper upon which the drawing, magnified in the same degree, has been made. This is the plan I have followed in all the drawings which illustrate my observations, and the scales have been copied in the wood-cuts and plates. All magnifying glasses of the same focus do not magnify in precisely the same degree, so that it is necessary for every observer to ascertain for himself the magnifying power of his lenses.

**65. Standards of Measurement.**—In this country we usually employ the English inch, but on the continent the Paris line =  $\cdot 0888$ , or about 1-11th of an English inch, is very generally used. The sign " is used to signify "of a line," and has been employed by Professor K  lliker in his works, while " signifies "of an inch."

**66. Conversion of Foreign Standards of Measurements.**—In order to compare the researches of different authors, it is often necessary to convert one expression of measurement into another. The accompanying table of Dr. Robertson's (*"Edin. Month. Jour. of Science,"* Jan. 1852) will be found of great use in making these calculations. Deputy Inspector General Lawson gives the following rules, in a paper communicated to my *"Archives"* (Vol. II, page 292). A unit is required that will admit of microscopic measurements being expressed in the smallest number of figures, and permit of foreign measures being easily converted into English, and *vice vers  *, and the decimal notation should be adopted to facilitate comparison between the measurements.

TABLE FOR MUTUAL CONVERSION OF BRITISH AND FOREIGN LINEAL MEASUREMENTS.

To convert—	1	2	3	4	5	6	7	8	9	Millimetres, Paris Lines, Prussian Lines,
1. British inches into Millimetres, . . . . .	25.39944	50.79988	76.19982	101.5992	126.9977	152.3973	177.7968	203.1963	228.5959	British Inches.
2. Do. Old Paris Lines . . . . .	28.51872	57.03744	85.55616	114.07488	142.59360	171.11232	199.63104	228.14976	256.66848	Paris Lines.
3. Do. Rhineland or Prussian Lines . . . . .	11.63275	23.26550	34.89825	46.53100	58.16375	69.79650	81.42925	93.06200	104.69475	Prussian Lines.
4. Millimetres into British Inches . . . . .	.03937079	.07874158	.11811237	.15748316	.19685395	.23622474	.27559553	.31496632	.35433711	British Inches.
5. Do. Old Paris Lines . . . . .	.44329	.88658	1.32987	1.77316	2.21645	2.65974	3.10303	3.54632	3.98961	Paris Lines.
6. Do. Rhineland or Prussian Lines . . . . .	.44878	.89756	1.34634	1.79512	2.24390	2.69268	3.14146	3.59024	4.03902	Prussian Lines.
7. Old Paris Lines into British Inches . . . . .	.088813	.177626	.266439	.355252	.444065	.532878	.621691	.710504	.799317	British Inches.
8. Do. Millimetres . . . . .	3.25866	6.51732	9.77598	13.03464	16.29330	19.55196	22.81062	26.06928	29.32794	Millimetres.
9. Do. Rhineland or Prussian Lines . . . . .	1.03194	2.06388	3.09582	4.12776	5.15970	6.19164	7.22358	8.25552	9.28746	Prussian Lines.
10. Rhineland or Prussian Lines into British Inches . . . . .	.083817	.167634	.251451	.335268	.419085	.502902	.586719	.670536	.754353	British Inches.
11. Do. Millimetres . . . . .	2.179704	4.359408	6.539112	8.718816	10.898520	13.078224	15.257928	17.437632	19.617336	Millimetres.
12. Do. Old Paris Lines . . . . .	.9652107	1.9304214	2.8956321	3.8608428	4.8260535	5.7912642	6.7564749	7.7216856	8.6868963	Paris Lines.

## Illustrations of Use of the above Table.

I.—EXAMPLE.		II.—EXAMPLE.		III.—EXAMPLE.	
Given 245.603 Paris Lines. Required the value in British Inches.		Given .00215 Millimetres. Required the value in British Inches.		Where extreme exactitude is not required, only one or two decimal places need be used. Thus—	
By line two of Table—		By line four of Table—		Given 2.826 British Inches. Required the value in Paris Lines.	
Old Paris Lines.	296	Millimetres.	20	By line two of Table—	
40	=	British Inches.	1	British Inches.	228.19
5	=	Millimetres.	20	Paris Lines.	11.36
9	=	British Inches.	20	Paris Lines.	9.91
.0033	=	British Inches.	20	Paris Lines.	.45
21.829631445 British Inches.		.000011685325 British Inches.		245.91 Paris Lines very nearly.	

Data, from which the Table has been calculated, extracted from Mr. Woodhouse's Table in the "Encyc. Metropolitana,"—British foot = 1. Metre = 3.280893.  
Old Paris foot = 1.0678. Rhineland or Prussian foot = 1.0408.



Most microscopic measurements are under the hundredth of an inch, and a hundred-thousandth of an inch cannot be measured with certainty. The requirements of the case therefore may be stated in decimals of an English inch by  $\cdot 00101$ , and if the two ciphers next the decimal point be struck out, and the first number be considered the unit, it may be written  $1^{\text{th}}01$ , in which a thousandth of an inch is the unit. This method will embrace nearly every microscopic magnitude in three consecutive figures.

The foreign measures are the millimetre and the French and Prussian lines. The two latter are so nearly equal, that in the small fraction required in the present subject they do not differ sensibly, and the same rule will serve for the conversion of both.

A millimetre contains  $\cdot 03937$  English inches or  $39^{\text{th}}37$ ; according to the method proposed, the length to be converted will seldom amount to one-fourth of this. To convert millimetres into thousandths, shift the decimal point one place to the right and multiply by 4; if greater accuracy be required, subtract  $1\frac{1}{2}$  from the second place of decimals for each of the nearest numbers of units of the product. Thus  $0^{\text{mm}}\cdot 250$  becomes  $2\ 50$  which  $\times 4 = 10^{\text{th}}00$ , from which subtract  $^{\text{th}}15$ ; and  $9^{\text{th}}35$  is obtained as the value in thousandths of an English inch, while  $0^{\text{mm}}\cdot 25$  is equal to  $9^{\text{th}}84$ , which differs from the former by a quantity too small to measure.

To convert thousandths of English inches into millimetres, add  $1\frac{1}{2}$  in the second place of decimals for the nearest number of units in the sum, divide by 4, and shift the decimal point one place to the left, thus—to  $9^{\text{th}}84$  add  $^{\text{th}}15$  and the sum  $9^{\text{th}}99 \div 4 = 2^{\text{mm}}\cdot 498$ , and shifting the decimal point  $^{\text{mm}}\cdot 2498$  which does not differ sensibly from  $^{\text{mm}}\cdot 25$ , the correct quantity.

A French line contains  $\cdot 0888$  English inches. To convert lines into thousandths of an inch, shift the decimal point one place to the right, and multiply by 9; if greater accuracy be required, subtract  $1\frac{1}{2}$  from the second place of decimals for each of the nearest number of units in the product. Thus  $0^{\text{line}}\cdot 125$  becomes  $1^{\text{th}}25$  which  $\times 9 = 11^{\text{th}}25$ , from which subtract  $^{\text{th}}14$ , and the value in thousandths is found to be  $11^{\text{th}}10$ , which is correct.

To count thousandths into lines add  $1\frac{1}{2}$  in the second place of decimals for each of the nearest number of units in the sum, divide by 9, and shift the decimal point one place to the left, thus,—to  $11^{\text{th}}10$  add  $^{\text{th}}14$ , the sum  $11^{\text{th}}25$  divided by 9, and the decimal point shifted one place to the left gives  $0^{\text{line}}\cdot 125$  as before.

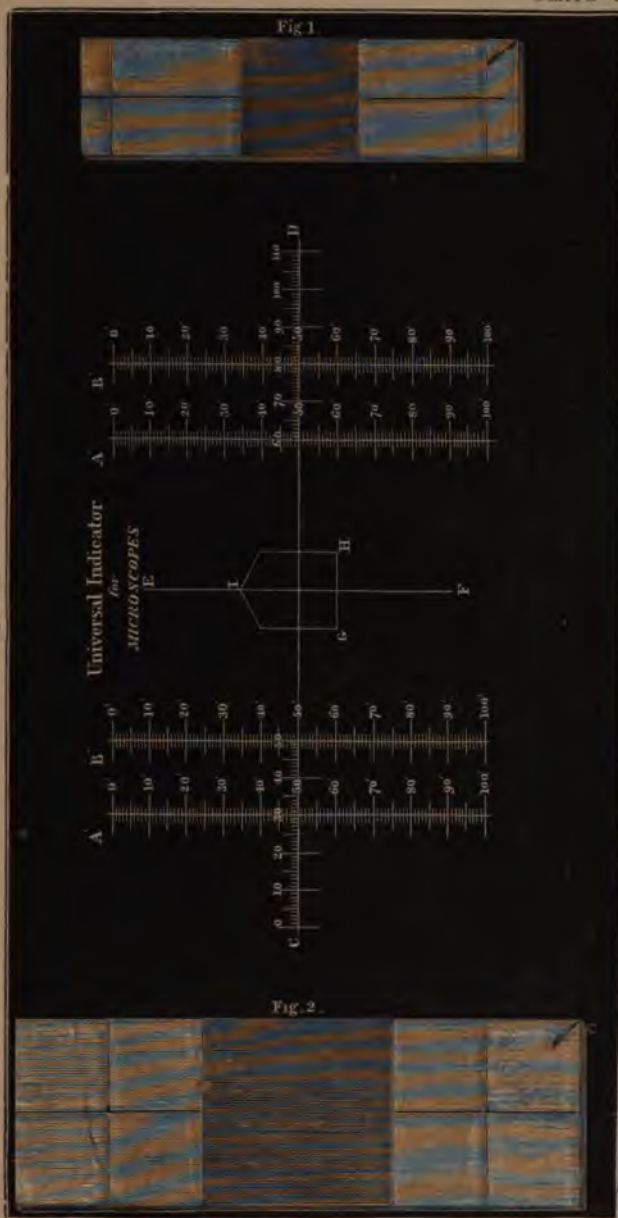
In most cases it will be unnecessary to apply the corrections noticed above, but by remembering the short rules given, any one on reading a foreign work may correct the measurements as he reads, and insert them in the margin without delay or interfering with his progress.

**67. On Finders.**—Various plans have been proposed from time to time for marking the exact position of a minute object in a specimen, so that it can be placed in the field of the microscope whenever required. A fine line of varnish or Brunswick black may be drawn round it, or a small and very thin metal tube (about the tenth of an inch in diameter) may be moistened with the varnish and pressed upon the glass cover, so as to encircle the particular object required with the line.

Mr. Bridgman, of Norwich, has designed an instrument for drawing a circle upon the thin glass with a diamond point (*"Microscopical Journal,"* Vol III, p. 237). This instrument is represented in Plate XVIII, Fig. 65. A, a brass cap fitting upon the end of the object glass, which it entirely covers up and protects from injury; B, a stem soldered to the side of the cap with the upper end having two projecting sides to steady the ends of C, *e*, and *f*, which are firmly secured to it; C, an elastic arm of hammered brass, which carries at its lower end D, a lever of thin brass plate, having a fragment of diamond inserted in its thinner end, and directly under the centre of the cap A; *c* and *f* are two springs, pressing upon the shorter end of the lever D, the longer one *f* has a hole to allow the screw *h* to pass without touching it; *g*, a screw holding the two springs and the elastic arm to the arm of the cap; *h*, a milled screw to adjust the elastic arm C, so as to bring the diamond point away from the centre, according to the size of the ring required. When the object has been found, the cap carrying the diamond point is placed on the object glass and carefully adjusted, so that the diamond point is brought into contact with the surface of the glass, it is then turned round, and thus a line is drawn round any object which can be readily found at any future time.

This same end has been gained in another manner. Graduated scales have been affixed to the stage of the microscope, so as to measure the exact amount of movement in the vertical and horizontal direction; the slide being placed in position against a stop at the side. The number on the two scales is noted when the

Fig. 67.



Bailey's Indicator. § 67.

[To face page 42.]





object is seen in the field, and, by placing the stage opposite the same numbers, at any future time the object must appear in the same position. Various ingenious "finders" have been proposed. A very simple and efficient one is represented in Plate XVIII, Fig. 64, in which the scales are ruled on paper (Mr Wright, "*Mic. Jour.*" Vol. I, p. 302, 1853), which is afterwards fixed upon the stage, but it is better to have the lines ruled on the brass itself.

*Bailey's Universal Indicator.*—Mr. J. W. Bailey, of the United States, has described an instrument for registering the positions of various objects upon a slide, in Vol. IV of the "*Quarterly Journal of Microscopical Science.*" The arrangement is seen in Plate XIX. The centre of the field of view corresponds to the point where the horizontal line C D intersects the vertical line E, F. The piece G, I, H is moveable. The slides upon which the objects are mounted must have guide lines, as shown in Figs. 1 and 2. This indicator is to be firmly fixed to the stage of the microscope, care being taken that the centre of the indicator corresponds to the centre of the object glass. The mode of using the indicator is obvious.

All such devices have, however, been superseded in cases where the microscope is provided with a travelling stage, by the very clever arrangement suggested by Mr. Maltwood ("*Trans. Mic. Soc.*" Vol. VI, p. 59, 1858). A little stop is placed upon one side of the stage, in contact with which one end of the finder, and afterwards the glass slide can be placed. The finder consists of a plate of glass, upon which numbers are arranged in minute squares. These run in two directions, vertically and horizontally, so that in each square there are two different numbers, except in the case of the central square, which of course contains two 25's. Any object having been found, its exact position may be registered by removing the slide and placing on the stage the finder. The numbers seen in the field are then marked on the slide itself, and the same spot can always be found, by looking for these numbers on the finder, moving the stage so that they come in the centre, and then substituting the slide for the finder. The numbers and lines are photographed on the finder which is made by Messrs. Smith and Beck, and costs 7s. 6d. A few of the squares of a Maltwood's finder are represented in Plate XVIII, Fig. 63.

**68. On Measuring the Angles of Crystals—Goniometers.**—*Measuring the Angles of Crystals.* I have already adverted to the

principal methods of measuring objects, but have not discussed the mode of ascertaining the value of the angles of microscopic crystals in the microscope. The simplest instrument for this purpose is the one represented in Fig. 68, Plate XX, which is a slight modification of *Schmidt's goniometer*. It consists of a cobweb stretched across the field of an eye-piece, and capable of being moved by an arm which passes round an accurately graduated arc. The cobweb line is placed parallel to one face of the crystal, the circle being moved round until the bar stands at zero. The latter is then made to rotate until the cobweb is brought parallel with another face. The number of degrees through which the bar has passed marks the angle of the crystal. It is absolutely necessary that in taking this measurement the crystal should be perfectly flat, for otherwise a false angle will be obtained. Dr. Leeson has proposed a beautiful and much more perfect arrangement for measuring the angles of small crystals. The plan has been improved by Mr. Highley, who describes, in the Fourth volume of the "*Quarterly Journal of Microscopical Science*," page 281, a mineralogical microscope. This instrument is represented in Plate XX, Fig. 71. It may thus be briefly described with the aid of the figure:—

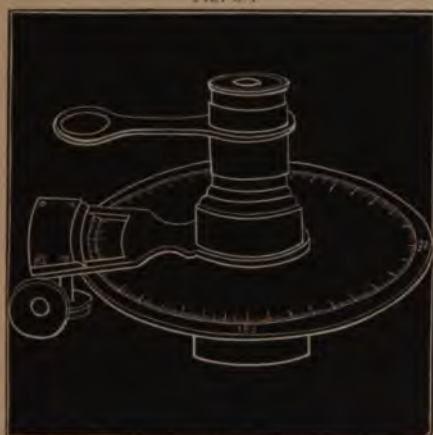
On a central pivot screwed into a solid circular base rotates a plate that carries the body, prism box P, object glass, and fine adjustment A: to the side of the base is fixed a square bar G, that carries the principal stage with its coarse adjustment, and the secondary stage into which fits the diaphragm polarizer, selenite plates, &c. A tube screws into the top of bar G, on which slides the mirror B. The body slides into a socket attached to the prism box at the proper angle, so that its axis will be perpendicular to the outer face of the prism P. Within the draw tube are fittings to receive glass tubes for examining with a Leeson's goniometer and minute stop, the amount of rotation in liquids that exhibit circular polarization.

A shorter body for other optical examinations replaces the ordinary one; this is fitted with a tourmaline T, and a cell for a plate of calc spar C, when the instrument is to be used as a modification of Professor Kobell's stauroscope for determining crystal systems; and two lenses LL with a Jackson's micrometer M, when the instrument is required for the determination of the optic axis on the principle of Soleil's instrument.

The prism P is contained in a solid brass box, on the upper



Fig. 69.



§ 68.

Fig. 69.



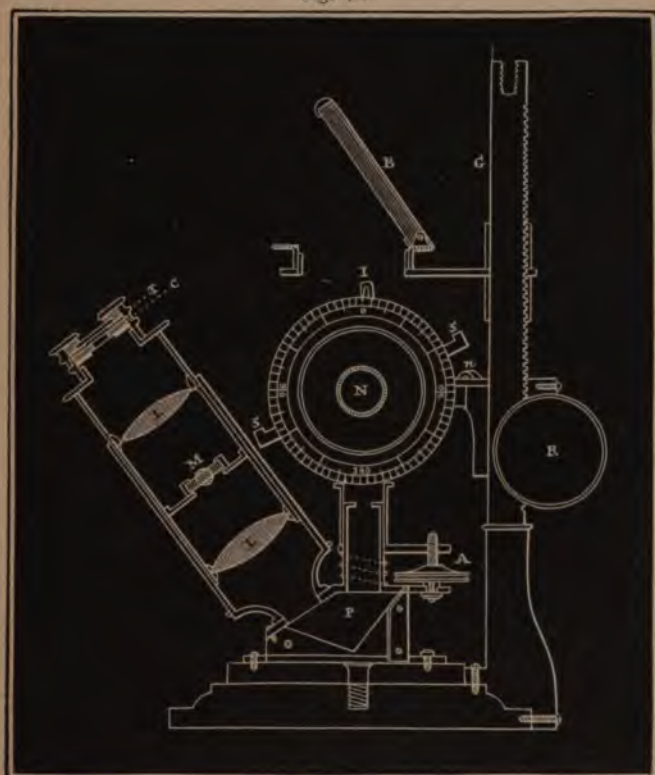
§ 35.

Fig. 70.



§ 23.

Fig. 71.



§ 65.

Fig. 68. Goniometer for measuring the angles of crystals in the microscope. Fig. 69. One of the crystals of Iceland spar, as arranged for the polariscope. Fig. 70. Crystals of oxalate of lime on a dark ground as seen by reflected light. Fig. 71. Dr. Leeson's arrangement for measuring the angles of small crystals, as constructed by Mr. Highley. This is described in page 44. [To face page 44.]



surface of which are screwed the tubes that carry the object-glass, and one side is removeable to allow of the prism being readily taken out and cleaned.

The fine adjustment consists of a tube screwed into the top of prism box at right angles to its surface, over this slides another tube on which the object-glasses are screwed. The spindle of the adjuster A rotates in a socket projecting from the prism box.

A semicircular arm works up and down the upright bar G, by means of a rack and pinion R, and supports the circular stage S, which is kept in a horizontal position by means of the nut N; the nuts N screwing on to the axes of the stage, clamp the stage firmly to the arm. The stage has a projecting ring, within which a graduated plate rotates when certain examinations have to be made: but which is ordinarily fitted with a plain metal plate that rises flush with the top of the axes of the stage. In this instrument the object has to be placed with the glass cover downwards.

to which a small retort-stand is fitted, may also be purchased of the instrument makers.

**70. Wire Retort Stands.**—Simple wire stands, made like retort-stands, which are fixed to a heavy leaden foot, will be found exceedingly useful little instruments to the microscopical observer. The rings can be readily raised or lowered at pleasure, and are well adapted to support light objects, such as glass slides over a lamp, test-tubes, flasks, and watch-glasses (Plate XXI, Fig. 72).

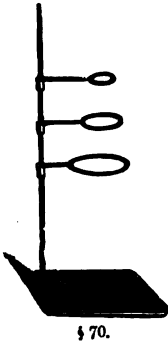
**71. Tripods** are made of thick iron wire, and are useful for supporting several pieces of apparatus used in microscopical research (Plate XXI, Figs. 75, 77).

**72. Brass Plate.**—The brass plate should be about six inches long by two broad, and about the thickness of thin millboard. It should be supported on three legs, of a convenient height for the spirit, or other lamp to be placed underneath, or the brass plate may be supported on one of the rings adapted to Mr. Highley's lamp. It is used for heating glass slides, in order to fix on the glass cells with the aid of marine glue, for mounting objects in Canada balsam, and for other purposes, where a uniform degree of heat is required to be applied to glass, which is very liable to crack if exposed suddenly to the naked flame. These different pieces of apparatus have been figured in Plate XXI, Fig. 74.

**73. The Water Bath** is of great use for drying objects previous to mounting them in Canada balsam. The object may be placed in a small porcelain basin, or large watch-glass, or it may be simply laid upon a flat plate. The basin or plate is then placed over the vessel containing water to which heat may be applied (Fig. 76). In order that vessels of different sizes may be heated upon the bath, it is convenient to have a few pieces of thin copper plate, with holes of different sizes cut in them, adapted for watch-glasses and small vessels (Fig. 76*a*). The advantage of drying by a steam heat consists in there being no danger of destroying the texture of the object by the application of too high a temperature. A water-bath may be very readily made by placing two porcelain basins one above the other, water

PLATE XXI.

Fig. 72.



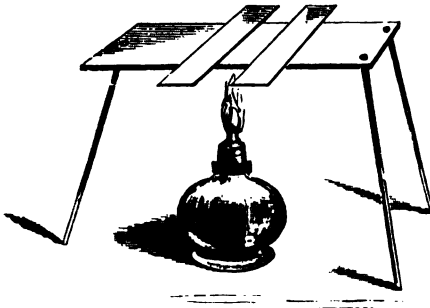
§ 70.

Fig. 73



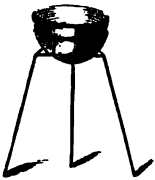
§ 69.

Fig. 74.



§ 72.

Fig. 75.



§ 73.

Fig. 76.



a



§ 73.

Fig. 77.

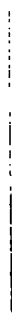


§ 71.

Fig. 72. Small retort-stand to support watch-glasses, &c.  
 Fig. 73. Spirit lamp with wire stand attached.  
 Fig. 74. Brass plate for heating glass slides.  
 Fig. 75. Porcelain basins arranged for a water bath.  
 Fig. 76. Small copper bath, with ring to diminish aperture.  
 Fig. 77. Tripod wire stand for supporting platinum basin containing matter for incineration.

[To face page 48.]





being poured into the lower one. These may be supported upon a tripod or upon one of the rings over the spirit lamp (Fig. 75).

#### INSTRUMENTS FOR CUTTING THIN SECTIONS OF TISSUES.

**74. Scalpels.**—It will be convenient to have three or four ordinary dissecting knives or scalpels for general use. One should be strong for the purpose of cutting hard substances.

**75. Double-edged Scalpels.**—For cutting thin sections, a knife of the form of a very narrow lancet will be found useful, and where only sections of small dimensions are required, this will answer all the purposes of Valentin's knife. In cases, however, where a section is wanted of considerable size, the latter instrument must be used. The double-edged scalpel should be very thin (Plate XXII, Fig. 81). Beautiful scalpels of this form are made by Messrs. Weiss, of the Strand. When employed for making a section, after cutting a clean surface, the point is made to perforate the surface, and carried along at a proper depth, so as to cut its way out. The width of the section may then be increased by carrying the knife from side to side.

**76. Section Knife of a New Form.**—A new section knife has been devised by Deputy Inspector General Lawson, for cutting very thin sections of soft tissues. The general form of the knife is represented in Plate XXIII, Figs. 87 and 88. It is fully described in my "*Archives*," Vol. III, p. 286.

**77. Double-bladed or Valentin's Knife.**—This instrument is of the greatest value in making thin sections of soft tissues, but care is required to keep it in good order. It is soon made blunt if used for cutting fibrous or cartilaginous textures. By its aid very beautiful sections of the kidney, liver, and other soft glandular organs may be obtained with the greatest facility. The blades should always be dipped in water or glycerine just before use, for, if wet, the operation of cutting is much facilitated, and the section more easily removed from between the blades. Immediately after use the blades should be washed in water, and dried with a soft cloth, or piece of wash leather. If a drop of water gets into the upper part of the knife where the blades meet, the

screw must be taken out, and each blade cleaned separately. With care in cleaning it, the knife may be kept in use a long time.

There are two forms of Valentin's knife ; in one the blades are sharp on both edges and of a lancet-shape, and in the other, which I much prefer, they are sharp at the point and wide at the base, so that the cutting edge slants downwards from the point, and they only cut on one side (Plate XXII, Fig. 82). The best form of Valentin's knife that I have used is that which has lately been made by Mr. Matthews (Fig. 83). The blades of this knife can be completely separated from each other and easily cleaned. Moreover the distance between the blades is regulated by a little screw, which is a most convenient arrangement. This knife has been further improved by Mr. Matthews, by the addition of two screws so that the perfect parallelism of the two blades is ensured.

**78. Razor.**—A strong knife made like a razor is very valuable for making sections of many tissues (Plate XXIII, Fig. 89).

**79. Scissors** are useful instruments for cutting small thin sections of different tissues. The most convenient form for this purpose is one in which the blades are curved, as in Fig. 78, Plate XXII. When only very small portions of a tissue are required for examination, they will be more readily removed with the scissors than with any other instrument. Several pair of scissors are required for microscopical purposes. Besides the ordinary form used for dissection, a small pair, with curved blades, a pair of very delicate scissors, with blunt points (Fig. 79), such as are employed for the dissection of insects, will be found of use. Some time since, I devised a new form of spring scissors, somewhat resembling the microtome. These are particularly well adapted for dissecting the nervous systems of insects, for following out the delicate ramifications of nerves and other minute dissections (Plate XXII, Fig. 80).

**80. Needles** of various sizes, form very useful instruments to the microscopical observer. They are employed for making minute dissections ; for tearing or unravelling various tissues, in order to display their elementary structure, and for separating any minute object from refuse or extraneous matter, previous to

Fig. 78.

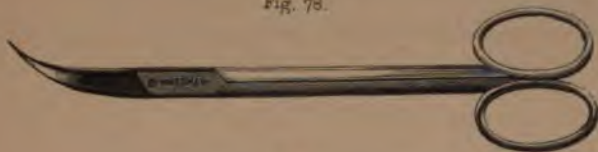


Fig. 79.

Fig. 79.

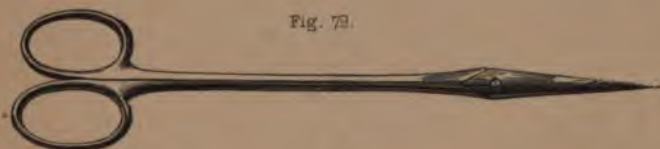


Fig. 80.

Fig. 80.



Fig. 81.

Fig. 81.



Fig. 82.

Fig. 82.



Fig. 83.

Fig. 83.



Fig. 84.

Fig. 84.

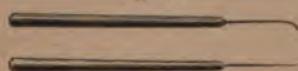


Fig. 85.

Fig. 78. Curved scissors, for cutting thin sections of tissues.

Fig. 79. Fine straight scissors, for dissecting. Fig. 80. Spring scissors, for making minute dissections.

Fig. 81. Double-edged scalpel, for cutting thin sections.

Fig. 82. Valentin's knife. Fig. 83. Valentin's knife, as improved by Mr. Matthews.

Fig. 84. Needles, for dissecting.

[To face page 50.]





its being mounted. Very thin needles are useful for separating substances under the field of the microscope. Needles which have been flattened at the points, and subsequently hardened, tempered, and sharpened on the two edges, make capital knives for very delicate work, or the pins used by the surgeons and termed *harelip pins* may be used with advantage. They may be inserted in a small wood stick (Fig. 84), or held in the handle of a crotchet needle. Mr. Matthews has lately made some needles with cutting edges, which are very useful for making minute dissections.

**81. Forceps.**—A pair of thin brass forceps will be found convenient for applying the thin glass cover after the preparation has been placed upon a slide or in a cell. A pair of dissecting forceps are also required by the microscopist. One pair should be strong with straight limbs, the other pair should be small, with thin curved blades, terminated with slightly rounded points, having very flat, but slightly roughened surfaces, of the pattern represented in Plate XXIII, Fig. 85.

Forceps for holding minute objects under the microscope are made to fix upon the stage (Fig. 86).

**82. Wooden Forceps** made of box-wood, with broad ends, are convenient for holding the glass slides when hot, for if held with cold metal forceps, the slides often crack. The same object may be gained more simply by fastening to the limbs of an ordinary pair of forceps, flat pieces of cork.

#### APPARATUS USED FOR EXAMINING OBJECTS IN THE MICROSCOPE.

**83. Plate Glass Slides**, the edges of which are ground and polished, may be obtained ready for use at six shillings per gross, or they may be easily cut out with the diamond, and the edges ground on the grinding slab. The slides now in common use in this country are three inches in length and one in breadth, and I cannot too strongly recommend the observer to employ slides of this size only for microscopical purposes. They should always be made of plate-glass, and pieces as clear as possible should be selected.

**84. Thin Glass.**—An object placed for examination upon a

glass slide is always protected with a piece of thin glass before it is placed upon the stage of the microscope. Thin glass now used for microscopical purposes is called cylinder glass, and is manufactured by Messrs. Chance of Birmingham. It may be obtained of different degrees of thickness. Thin glass in sheets should be kept in fine sawdust, as it is very readily broken, in consequence of being imperfectly annealed. When cut up in small pieces, it should be kept in a little box, with a little powdered starch, which prevents the pieces being broken. For cutting the thin glass an instrument termed a *writing diamond* is employed, and this is also used by some observers for writing the name of the preparation upon the glass slide. As a general rule, however, I think it better to write the name of the specimen upon a small label which can be gummed to the glass.

*Glass Cells* are described in Chapter IV. Thin glass of various degrees of thickness, and already cut into squares and circles, may be obtained of Messrs. Claudet and Houghton, High Holborn. For the very high powers the thinnest pieces must be selected from a considerable quantity. Messrs. Powell and Lealand supply the thin glass for use with their twenty-fifth.

**85. Watch Glasses** of various sizes should be kept by every observer, as they are convenient for many purposes. They cost about a shilling per dozen, and may be obtained of the watch-makers. The lunette glasses are useful for examining substances in fluids with low powers, as in these we are enabled to obtain a considerable extent of fluid of nearly uniform depth.

The little porcelain moulds in which moist colours are kept, and the little circular and oval shallow dishes, are most useful for soaking microscopical specimens in various solutions prior to examination or mounting. They may be covered by circular pieces of glass.

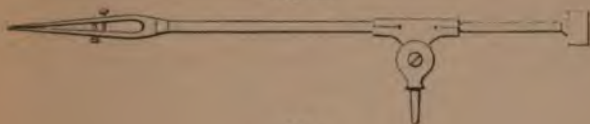
**86. Glass Shades.**—Every microscopist should be provided with from six to twelve small glass shades from two to four or five inches in diameter, to protect objects which are being mounted from the dust. The cheap slightly green propagating glasses, now commonly sold at all the glass shade shops, are most convenient for this purpose. They cost from 2s. to 5s. per dozen. These shades are figured in Plate XXIII, Fig. 92.

Fig. 85.



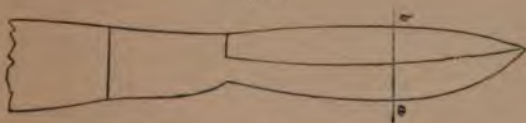
§ 81.

Fig. 86.



§ 81.

Fig. 87.



§ 76.

Fig. 89.



§ 78.

Fig. 90.



Fig. 91.



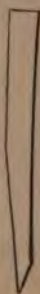
§ 94.

Fig. 92.



§ 169.

Fig. 88.



§ 76.

Fig. 85. Curved forceps, for minute dissection.

Fig. 86. Forceps which can be attached to the stage of the microscope, for holding objects during examination.

Fig. 87. New form of section knife. Fig. 88. Section of the same through a, b.

Fig. 89. Fine strong knife, for cutting thin sections of hard tissues.

Fig. 90. Fine saw, for sawing bone and other hard tissues.

Fig. 91. Vessel for containing Canada balsam, gum, cements, &c.

Fig. 92. Glass shades, for protecting objects from dust while being mounted.

[To face page 52.]





## CEMENTS.

The chief cements employed in microscopical work, are *Gold size*, *Sealing-wax varnish*, *Solution of shell-lac*, *Solution of asphalt*, *Marine glue*, *Canada balsam*, *Gum*, and a *French cement* composed of lime and India-rubber. These cements are used for attaching the glass cell to the glass slide, for fixing the cover upon the preparation after it has been properly placed in the cell, and for other purposes. The liquid cements should be kept in very wide-mouthed bottles, or in a capped bottle (Plate XXIII, Fig. 91).

**87. Gold Size** is prepared by melting together gum animi, boiled linseed oil, red lead, litharge, sulphate of zinc, and turpentine. Gold size adapted for microscopical purposes may be also prepared as follows:—25 parts of linseed oil are to be boiled with one part of red lead, and a third part as much umber, for three hours. The clear fluid is to be poured off and mixed with equal parts of white lead and yellow ochre, which have been previously well pounded. This is to be added in small successive portions, and well mixed; the whole is then again to be well boiled, and the clear fluid poured off for use. In this country it may be obtained at any varnish makers.

**88. Sealing-wax varnish** is easily made by dissolving the best sealing wax of any colour which may be desired, in tolerably strong alcohol. This cement is, however, apt to dry rather brittle, and should not, therefore, be used in cases where it is of the greatest importance to keep the cell perfectly air-tight. It forms a good varnish for the last coat. Various colours may be kept according to taste.

**89. Solution of Shell-lac** is recommended by Mr. Ralphs for fixing down the thin glass cover. It is made by dissolving shell-lac in spirits of wine. The shell-lac should be broken in small pieces, placed in a bottle with the spirit, and frequently shaken, until a thick solution is obtained. It dries rapidly, and, if put on in thin layers successively, forms a good cement. It is not acted upon by weak spirit.

**90. Bell's Cement.**—The best cement for specimens immersed



in glycerine is sold by Messrs. Bell, chemists, Oxford-street. This, I believe, was originally suggested by Mr. Tomes, but I do not know its exact composition. It appears to contain shell-lac and gold-size.

**91. Brunswick Black.**—Solution of asphalt in turpentine commonly known by the name of Brunswick black, may be obtained at any oil-shop, and forms a most useful cement, both for making very thin cells, and also for fixing on the thin glass covers. If a little solution of India-rubber in mineral naphtha be added to it, there is no danger of the cement cracking when dry. For this hint I have to thank my friend, Mr. Brooke. I have many preparations which have been cemented with Brunswick black which have been kept for upwards of ten years. It is always desirable, however, to paint on a new layer from time to time, perhaps once in twelve months.

Common Brunswick black is made by melting one pound of asphaltum, and then adding half a pound of linseed oil, and a quart of oil of turpentine. The best Brunswick black is prepared by boiling together a quarter of a pound of foreign asphaltum, and four and a quarter ounces of linseed oil, which has been previously boiled with half an ounce of litharge until quite stringy; the mass is then mixed with half a pint of oil of turpentine, or as much as may be required to make it of a proper consistence. It is often improved by being thickened with lamp black. It must be remembered that this cement is soluble in oil of turpentine.

**92. Marine Glue.**—This substance was, I believe, first used for microscopical purposes by Dr. Goadby, of Philadelphia. It is prepared by dissolving, separately, equal parts of shell-lac and India-rubber, in coal or mineral naphtha, and afterwards mixing the solutions thoroughly with the application of heat. It may be rendered thinner by the addition of more naphtha. Marine glue is readily dissolved by naphtha, ether, or solution of potash. It is preserved well in a tin box. I shall describe the manner of using marine glue and the different cements I have alluded to, in Chapter IV.

**93. Cement for attaching Gutta Percha or India-rubber to the Glass Slides.**—A cement for attaching cells of gutta percha

or India-rubber to the glass slide may be made as follows:—According to Harting, gutta percha is to be cut into very small pieces and stirred, at a gentle heat, with fifteen parts of oil of turpentine; the gritty, insoluble matter, which the gutta percha always contains, is to be separated by straining through linen cloth, and then one part of shell-lac is to be added to the solution, kept at a gentle heat, and occasionally stirred. The mixture is to be kept hot until a drop, when allowed to fall upon a cool surface, becomes tolerably hard. When required for use, the mixture is to be heated, and a small quantity placed upon the slide upon which the cell is to be fixed; the slide itself is then to be heated.

**94. Canada Balsam** is much employed by microscopical observers: formerly it was used for cementing cells together, but this is now effected more readily by the aid of marine glue.

Canada balsam is a thick viscid oleo-resin, which becomes softer upon the application of a gentle heat. If it be exposed to too high a temperature, the volatile oil is expelled, and a hard brittle resin remains behind. It is chiefly employed for mounting hard dense textures; and, in consequence of its great power of penetrating textures, and its highly-refracting properties, the structure of many substances, which cannot be made out by the ordinary mode of examination, is rendered manifest by this medium. Canada balsam should be preserved in a tin box, care being taken to exclude the dust; or in a bottle having a cap to it. The balsam should be kept very clean, otherwise preparations mounted in it will often be spoiled in consequence of the accidental introduction of foreign bodies. It has been frequently recommended that the oldest specimens of balsam should alone be employed for microscopical examination. By exposure to the air, the balsam becomes very thick, and unfit for use: it may be thinned by the addition of turpentine, but this should always be avoided as it renders the balsam liable to become streaky some time after the preparation has been mounted, and bubbles are often found in it. It is, however, always better to use balsam which has been kept in well-closed vessels (Plate XXIII, Fig. 91) or in a tin pot.

**95. Of Vessels for Keeping Canada Balsam in.**—The tubes, made of thick tin-foil, used for artists' colours, with a small cap

that screws on to the top, as has been suggested by Mr. Suffolk, are very convenient receptacles for the preservation of Canada balsam. As they contain no space for air, the balsam does not become hard and unmanageable, as is too often the case when it is kept in bottles or tin pots. There is no necessity for using a glass or metal rod, as the quantity of balsam required can always be forced out without the slightest difficulty. Other cements and varnishes can be kept in them also for any length of time. It is as well, however, to keep them in an upright position, to prevent the cement from running into the thread of the screw, and so fixing the top too tightly.

**96. Solutions of Canada Balsam.**—Canada balsam is soluble in ether, but the best solvent is chloroform. Many very delicate structures may be mounted in Canada balsam, by immersing them in a chloroform solution. As the chloroform evaporates the balsam becomes stronger.

**97. Gum.**—Thick gum-water will be found very useful for attaching labels to preparations, and also for fixing on the cover when preparations are mounted in the dry way. It is prepared by placing common gum-arabic in cold water, and keeping the bottle in a warm place until the solution has become sufficiently thick. It should always be strained before it is placed in the bottle for use.

Gum-water, thickened with powdered starch or whiting, is a very useful cement for fixing the glass cover on preparations mounted dry. When dry it forms a hard white coating. The addition of a little arsenious acid will prevent the growth of mildew. Another very convenient solution is made by dissolving powdered gum in a weak solution of acetic acid.

**98. French Cement composed of Lime and India-rubber.**—The French cement composed of lime and India-rubber is very valuable for mounting all large microscopical preparations. The principal advantages are, that it never becomes perfectly hard, and it therefore permits considerable alteration to take place in the fluid contained in the cell without the entrance of air, it also adheres very intimately to glass, even if it be perfectly smooth and unground. Suppose a glass cover is to be affixed to a large cell containing fluid. A small piece of the cement is taken be-



tween the finger and thumb and carefully rolled round until it can be drawn out into a thread about the eighth or tenth of an inch in thickness. I apply this to the top of the cell, before introducing any fluid, and slightly press it down with the finger previously moistened. It adheres intimately. The preservative fluid with the preparation are now introduced and the cell filled with fluid which indeed is allowed to rise up slightly above its walls. The glass cover, rather smaller than the external dimensions of the cell, and slightly roughened at the edges, is to be gently breathed upon, and then one edge is applied to the cement, so that it may be allowed to fall gradually upon the surface of the fluid which is now seen to wet each part of the cover successively, until it completely covers the cell, and a certain quantity of the superfluous fluid is pressed out. By the aid of any pointed instrument a very little cement is removed from one part, so that more fluid may escape as the cover is pressed down gently into the cement. The pressure must be removed very gradually, or air, of course, will enter through the hole. A bubble of air entering in this manner may often be expelled again by pressure, or it may be driven out by forcing in more fluid through a very fine syringe at another part of the cell; but it is far better to prevent the entrance of air in the first instance. The edge of the glass cover being thoroughly embedded in the cement, the small hole is to be carefully plugged up by a small piece of cement, and the cell allowed to stand perfectly still for a short time, when it may be very gently wiped with a soft cloth. The edges of the cement may be smoothed by the application of a warm iron wire, and any superabundance removed with a sharp knife. A little Brunswick black or other liquid cement may be applied to the edges, for the purpose of giving the whole a neater appearance.

The cement is made as follows :—A certain quantity of India-rubber scraps is carefully melted over a clear fire in a covered iron pot. The mass must not be permitted to catch light. When it is quite fluid, lime, in a perfectly fine powder, having been slacked by exposure to the air, is to be added by small quantities at a time, the mixture being well stirred. When moderately thick, it is removed from the fire and well beaten in a mortar and moulded in the hands until of the consistency of putty. It may be coloured by the addition of vermilion or other colouring matter. I have several preparations which have been

placed in the creosote and naphtha solution in large cells, and they are now perfectly air-tight, although upwards of seven years have elapsed since they were first put up. The lime and India-rubber cement answers well for fixing on the glass tops of large preparation jars, and looks very neat; but, if moderately strong spirit be used, a little air must be permitted to remain in the jar.

#### PRESERVATIVE SOLUTIONS.

99. **Spirit and Water**—Spirit and water forms a well known and valuable medium for preserving anatomical preparations. In diluting spirit, distilled water only should be employed; for if common water be mixed with spirit, a precipitation of some of the salts dissolved in it not unfrequently takes place, which renders the mixture turbid and unfit for use. Proof spirit will be strong enough for all general purposes, except for hardening portions of the brain or nervous system, when stronger spirit must be used. Two parts of rectified spirit, about sp. gr. 837, mixed with one part of pure water, makes a mixture of sp. gr. 915-920, which contains about 49 per cent. of real alcohol, and will therefore be about the strength of proof spirit. One part of alcohol, sixty over proof, to five parts of water, forms a mixture of a sufficient strength for the preservation of many substances, and many microscopical specimens may be preserved in a solution more diluted than this. Within the last few years, the Government has permitted the use of methylated alcohol for various purposes in the arts, which pays no duty. This spirit answers well for preserving anatomical preparations, and is a great boon to all engaged in putting up large anatomical specimens. It may be obtained at the price of 5s. 6d. a gallon, sixty degrees over proof, of Messrs. Lightly and Simon, and of other distillers, in quantities of not less than ten gallons at a time.

In the first instance, application must be made to the Board of Inland Revenue, Somerset House, for permission to use the spirit, by letter, accompanied with the names of two respectable householders, who are willing to act as bond that the applicant only uses it for the purposes stated in his application. The probable quantity required annually must also be stated.

100. **Glycerine**.—This is one of the most valuable fluids ever



employed for microscopical purposes. I believe Mr. Warington, of Apothecaries' Hall, was the first observer who used this medium as a preservative fluid.

A solution of glycerine adapted for preserving many structures is prepared by mixing equal parts of glycerine with camphor water. The latter prevents the tendency to mildew, or it may be mixed with naphtha and water, or with the creosote solution to be described presently. The degree of dilution will depend upon the nature of specimen. If the substance be at all opaque it will be necessary to employ strong glycerine. I have many preparations which have been preserved in glycerine for nearly twenty years. Of the importance of glycerine, as a preservative fluid, I shall have to speak in Chapter VI.

For preserving medusæ and delicate marine animals Dr. Carpenter recommends a solution composed of *sea water* with one-tenth of *alcohol* and the same quantity of glycerine.

Glycerine is obtained by boiling oil with litharge. The oleate of lead remains as an insoluble plaster, while the glycerine is dissolved. It may be rendered free from lead by passing a current of sulphuretted hydrogen through it; and the clear solution, after filtration, may then be evaporated to the consistence of a syrup.

The glycerine which is now distilled by a patent process, and known as Price's glycerine, is much superior to the ordinary glycerine. It is perfectly colourless, free from all impurities, and of much greater density. The specific gravity of Price's patent glycerine is 1240, while the common is only 1196·6. The former costs about 6s. and the latter 2s. 6d. a pound.

For twelve years I have used glycerine for preserving almost every structure. In Chapter X will be found the results of my most recent experience of this substance, from the use of which I have learnt more than from any other preservative medium.

**101. Thwaites' Fluid.**—This fluid has been much employed by Mr. Thwaites for preserving recent specimens of desmidiæ; but it is also applicable to the preservation of a vast number of animal substances.

It is made as follows:—

Water . . . . .	16 ounces.
Spirits of wine . . . . .	1 ounce.
Creosote, sufficient to saturate the spirit.	
Chalk, as much as may be necessary.	

Mix the creosote and spirit, stir in the chalk with the aid of a pestle and mortar, and let the water be added gradually. Next add an equal quantity of water saturated with camphor. Allow the mixture to stand for a few days and filter. In attempting to preserve large preparations in this fluid, I found that it always became turbid, and therefore was led to try several modifications of it. The solution next to be described was found to answer very satisfactorily.

Water may also be impregnated with creosote by distillation. It should be remarked that M. Strausdurkheim has succeeded in preserving animal preparations in camphor water only.

#### 102. Solution of Naphtha and Creosote<sup>6</sup>

Creosote . . . .	3 drachms.
Wood naphtha . . . .	6 ounces.
Distilled water . . . .	64 ounces.
Chalk, as much as may be necessary.	

Mix first the naphtha and creosote, then add as much prepared chalk as may be sufficient to form a smooth thick paste; afterwards add, very gradually, a small quantity of the water, which must be well mixed with the other ingredients in a mortar. Add two or three small lumps of camphor, and allow the mixture to stand in a lightly-covered vessel for a fortnight or three weeks, with occasional stirring. The almost-clear supernatant fluid may then be poured off and filtered if necessary. It should be kept in well-corked or stoppered bottles.

I have some large preparations which have been preserved in upwards of a pint of this fluid, for more than twelve years, and the fluid is now perfectly clear and colourless. Some dissections of the nervous systems of insects have kept excellently; the nerves retain their white appearance, and have not become at all brittle. Two or three morbid specimens are also in an excellent state of preservation, the colour being to a great extent preserved, and the soft character of the texture remaining. I have one preparation mounted in a large gutta percha cell, containing nearly a gallon of this fluid.

A solution of wood naphtha or pyroacetic spirit, in water, has been recommended by Professor Quekett, and forms an excellent preservative solution, in the proportion of one part of the naphtha to ten of water. The solution is often a little cloudy, but may be made quite clear by filtration after the mixture has been allowed to stand still for some days.

One great advantage of these aqueous preservative solutions is that the natural appearance of the structure is very slightly altered. The solution, however, after a time, renders many of the more delicate structures more or less granular.

**103. Carbolic Acid.**—A solution of carbolic acid in distilled water also preserves many animal and vegetable preparations exceedingly well. The water will only take up a very small quantity, but the preservative properties of the weakest solution are very great.

**104. Solution of Chromic Acid.**—A solution of chromic acid is well adapted for preserving many microscopical specimens. It is particularly useful for hardening portions of the nervous system previous to cutting thin sections. The solution is prepared by dissolving sufficient of the crystallized acid in distilled water to render the liquid of a pale straw colour.

The crystallized acid may be prepared by decomposing 100 measures of a saturated solution of bichromate of potassa, by the addition of 120 to 150 measures of pure concentrated sulphuric acid. As the mixture becomes cool, crystals of chromic acid are deposited, which should be dried and well pressed on a porous tile, by which means the greater part of the sulphuric acid is removed, and the crystals obtained nearly pure.

**105. Preservative Gelatine.**—This substance was first employed for preserving microscopical textures by Mr. H. Deane, who gives the following directions for its preparation :—

Gelatine	.	.	.	.	1 ounce.
Honey.	.	.	.	.	4 ounces.
Spirits of wine	.	.	.	.	$\frac{1}{2}$ ounce.
Creosote	.	.	.	.	6 drops.

Soak the gelatine in water until soft, and to it add the honey which has been previously raised to the boiling-point in another vessel. Next, let the mixture be boiled, and after it has cooled somewhat, the creosote dissolved in the spirits of wine is to be added. Lastly, filter through thick flannel to clarify it.

When required for use, the bottle containing the mixture must be slightly warmed, and a drop placed on the preparation upon the glass slide, which should also be warmed a little.



Next, the glass cover, after having been breathed upon, is to be laid on with the usual precautions, and the edges covered with a coating of the Brunswick black varnish. Care must be taken that the surface of the drop does not become dry before the application of the glass cover; and the inclusion of air-bubbles must be carefully avoided.

**106. Gelatine and Glycerine.**—A mixture of gelatine and glycerine makes a very valuable medium for preserving different animal and vegetable structures.

The mixture may be made as follows:—A certain quantity of gelatine or isinglass is allowed to soak for some time in cold water, until it swells up and becomes soft. It is then placed in a glass vessel and melted by the heat of warm water. It may be clarified if necessary by first adding to the cool gelatine a little white of egg, then boiling the mixture, and filtering through fine flannel. To this fluid, an equal quantity of strong glycerine is added and well mixed with it. This mixture may be kept for any length of time, and a very slight heat is sufficient to render it perfectly fluid.

**107. Gum and Glycerine.**—Mr. Farrauts has suggested the following preservative medium which will be found most useful for mounting very many objects:—

Picked gum Arabic	.	.	.	4 ounces by weight.
Distilled water	.	.	.	4       "       "
Glycerine	.	.	.	2       "       "

It is to be kept in a stoppered bottle and a piece of camphor added to the solution.

**108. Goadby's Solution.**—This is made of several different strengths. That most generally useful is the following:—

Bay salt	.	.	.	4 ounces.
Alum	.	.	.	2 ounces.
Corrosive sublimate	.	.	.	4 grains.
Boiling water	.	.	.	4 pints.

Mix and filter. This solution for most purposes may be diluted with an equal bulk of water. For preserving delicate preparations it should be even still more dilute. Goadby's solution is very valuable for preserving many anatomical

specimens, but as it tends to render tissues hard and opaque, it is not adapted for the preservation of many structures which are to be examined in the microscope.

109. **Burnett's Solution**, consisting of chloride of zinc, is a powerful antiseptic, but not adapted for the preservation of microscopical specimens.

110. **Chloride of Calcium**.—A saturated aqueous solution of chloride of calcium, free from iron, has been much recommended for preserving specimens of bone, hair, teeth, and other hard structures, as well as many vegetable tissues. A solution of chloride of calcium has been used by the late Professor Schröder Van der Kolk, of Utrecht, for keeping sections of the spinal cord and preparations of nerves. Many of these, through the kindness of my friend, I had an opportunity of seeing and can testify to their excellence.

111. **Alum**.—A solution of *alum* in the proportion of one part of alum to sixteen of water has been found to answer pretty well for some substances. Gannal's solution, which consists of one part of *acetate of alumina* dissolved in ten parts of water; solutions of *common salt* (one part to five of water, with a little camphor), *corrosive sublimate*, *persulphate of iron*, *sulphate of zinc*, and solutions of several other salts, have been recommended as preservative solutions, but although adapted for the preservation of animal substances, they cannot be employed for microscopical specimens, in consequence of their tendency to render the textures very opaque and granular.

112. **Arsenious Acid** has been much recommended, and my friend Dr. Andrew Clarke has preserved many beautiful specimens of lung tissue and other structures in an aqueous solution of this substance.

113. **Arseniuretted hydrogen gas** has also been recommended for the preservation of animal substances, but it is not adapted for microscopical preparations. Dr. Richardson has lately kept animal substances from decomposition by immersing them in an atmosphere of *nitrogen*, which is prepared by placing a piece of phosphorous in a stone jar containing common air, and provided



with an air-tight cover. The oxygen is soon exhausted, and no decomposition can take place.

Most of the preservative solutions which I have described may be obtained of Mr. Highley, Green Street, Leicester Square. The mode of using these will be described in Chapter VI. Every microscopist engaged in any special inquiry will of course alter the composition of these solutions in any way experience may show him to be advisable. Great improvements doubtless may yet be made in many preservative solutions. A series of exact experiments of the effects of the different fluids upon the same textures is much to be desired, and this is one of the questions upon which amateurs might contribute most valuable information.

## CHAPTER IV.

ON THE METHODS OF MAKING CELLS FOR MICROSCOPICAL PREPARATIONS. — *Cells for Preserving Microscopical Specimens—Cells for Dry Objects—Paper Cell—Holder for Pressing Objects, &c.—Brunswick Black Cell—Cells made of Tinfoil and Marine Glue—Of Cutting and Grinding Glass—Of Cutting the Thin Glass—Stone or Pewter Slab for Grinding Glass—Cementing Glass together with Marine Glue—Cleaning off Superfluous Glue—Cells made of Thin Glass—Simple Methods of Perforating the Thin Glass—Deeper Glass Cells—Small deep Glass Cells for Injections—Built Glass Cells—Deep Glass Cells made by Bending a Strip of Glass in the Blowpipe Flame—Moulded Glass Cells—Gutta Percha—Troughs for Examining Zoophytes—Animalcule Cages—Round Cells Proposed by Dr. Guy.*

## CELLS FOR PRESERVING MICROSCOPICAL SPECIMENS.

ALL objects intended for microscopical observation should be protected with a cover of thin glass. This cover prevents the entrance of dust, and protects the object from exposure to the atmosphere. The fluid in which many objects are placed for examination would rise in vapour which would condense upon the object-glass, and give rise to great inconvenience were it not prevented from evaporating by a thin glass cover. If the thin glass, however, should press upon the object placed upon the glass slide, its distinctness will be impaired, or the structure may be entirely destroyed—an inconvenience which is prevented by placing some substance slightly thicker than the object with it between the glasses. If this entirely surround the object, a little cavity is made in which a specimen may be placed, and afterwards covered with thin glass without risk of injury from pressure. This is termed a cell.

Cells may be composed of various materials according to the thickness which may be necessary, or according to the nature of the substance to be placed within them.

**114. Paper Cells.**—For *dry objects* an efficient cell is readily made with a ring of paper or cardboard fixed with gum to the glass slide; or a hole may be punched out of a piece of cardboard, wood, millboard, or gutta percha, or a vulcanized India-rubber ring may be cemented to a slip of glass. Many other devices will occur to the mind of any one who wishes to make neat cells of this kind. If, however, the cell is intended to contain fluid, it must be made of some substance impervious to moisture.

**115. Holder for Pressing Objects between Glasses, &c.**—In mounting objects, it is often requisite to subject them to firm pressure between two of the glass slides. The pressure may be obtained by the aid of weights or screws, or by the very simple and efficient arrangement devised by Mr. Gorham. (See Plate XXIV, Fig. 97.)

**116. Brunswick Black Cell.**—A very thin cell may be made by painting a ring of Brunswick black or gold size upon the glass slide, and then allowing it to dry.

The best form of Brunswick black cell is the circular one, which is so easily made by the aid of Mr. Shadbolt's excellent apparatus (Plate XXIV, Fig. 93). The slide is placed on the little brass wheel which is made to revolve, while a brushful of Brunswick black is held at the proper distance from the centre, according to the diameter of the cell required. If a thick layer is desired the slide may be warmed, when the layers of Brunswick black applied dry very quickly.

**117. Marine Glue Cells** may be made according to the same plan. In order to make such a cell, a glass slide is warmed upon the brass plate (§ 72), and when hot enough a small piece is allowed to melt upon the slide, and moved round and round in the position in which the wall of the cell is to be. When the glue has been allowed to cool, any superfluity may be removed from the slide with a sharp knife.

**118. Cells made of Tinfoil.**—A piece of tinfoil may be cut out,

PLATE XXIV.

Fig. 93.



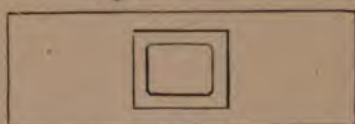
§ 116.

Fig. 94.



§ 121.

Fig. 96.



§ 124.

Fig. 94.\*



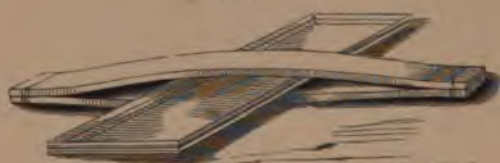
§ 123.

Fig. 95.



§

Fig. 97.



§ 115.

Fig. 98.



§ 119.

Fig. 99.



§ 120.

- Fig. 93. Shadbolt's apparatus for making round cells of Brunswick black.  
 Fig. 94. Flat brass rings for cutting circles of thin glass.  
 Fig. 94\*. Large bradawl, for scraping away superfluous marine-glue in making cells.  
 Fig. 95. Plate glass stage for examining objects when immersed in acids or corrosive liquids.  
 Fig. 96. Thin glass cell, for examining deposits from fluids.  
 Fig. 97. Holder, constructed of two pieces of whalebone, tied together, or riveted at both ends.  
 Fig. 98. To illustrate the manner in which the diamond is used for cutting thin glass.  
 Fig. 99. Writing diamond for cutting thin glass.

[To face page 66.]





so as to form a slightly thicker cell, and may be fixed upon the slide with marine glue, as in Fig. 96, Plate XXIV.

**119. Cutting and Grinding Glass.**—In the manufacture of cells presently to be described, glass is required to be cut with a diamond and ground perfectly smooth at the edges. Moderately thick glass is cut with the ordinary glazier's diamond (Plate XXIV, Fig. 98), but when we require to cut plate glass, a larger diamond than that in ordinary use is necessary.

**120. Cutting Thin Glass.**—The *thin glass* is cut with the writing diamond (Plate XXIV, Fig. 99), which makes a scratch sufficiently deep to permit of the glass being broken off very smoothly.

The *circles* of thin glass may be cut by carrying the diamond round openings which have been turned in pieces of brass. Of these many different sizes may be made so that circular pieces of thin glass of any required diameter, may be easily obtained (Fig. 94).

**121. Stone for Grinding.**—Glass can be *ground* upon a perfectly *flat stone* with emery powder or fine sand and a little water, or, instead of the stone, a flat *plate* composed of pewter may be used, as was recommended by Dr. Goadby. The emery after a time becomes embedded in the pewter, and thus a very efficient surface for grinding is obtained.

The pewter plate may be cast in the form of a flat circular disk, which can be placed upon a pivot and made to revolve rapidly in a horizontal direction by means of a multiplying winch connected with it—an arrangement which is desirable when it is important to save labour as much as possible.

**122. Cementing Glass together with Marine Glue.**—The surface of glass to which a cement is to be applied should always be roughened by grinding, as the cement adheres much more intimately to a rough surface than to the polished glass.

Glass is cemented together with marine glue, and in making large built glass cells, the edges are united by means of the same substance, which can now be readily obtained. Formerly gold size, Canada balsam, and other cements were employed, but these are all inferior to marine glue.

The manner of applying the marine glue to the glass has been

already alluded to. The glass must always be warmed upon a flat brass or iron plate, so that the heat may be applied gradually and equally. It must not be touched with cold fingers, but must be held with wooden forceps, or with ordinary forceps, the extremities of which have been protected with pieces of cork, in the manner described in § 82.

When the pieces of glass of which the cell is to be composed are warm enough, a little glue cut into small pieces is allowed to melt in the position in which the glass is to be fixed. When it is melted, the glass is applied and pressed down upon a deal board, so as to squeeze out as much marine glue as possible.

**123. Cleaning off Superfluous Glue.**—While the slide is yet warm, much of the glue may be scraped off with an old knife and small chisel (Plate XXIV, Fig. 94\*), after which a little *solution of potash* (the *liquor potassæ* of the shops) will soften the remainder. It may then be very readily removed with the aid of soap and water and a nail brush. Or the whole cell may be soaked in equal parts of liquor potassæ and water,—but we must bear in mind that if the cell be soaked for too long a time in strong solution of potash, there is danger of the glue between the glass being softened. The potash must always be carefully washed away, to prevent the chance of the glue being softened after the cell is complete.

**124. Cells made of Thin Glass.**—The neatest and most perfect *shallow cell* is formed by making a hole of the required size in a piece of thin glass. This used to be effected as follows:—Many pieces of thin glass were glued together with marine glue, and when cold a hole was drilled through them all. Lastly they were separated from each other by heat, and cleaned with potash in the usual manner.

**125. Simple Methods of Perforating the Thin Glass.**—Thin glass cells may, however, be readily made by every microscopist for himself, according to either of the following plans:—My friend, Dr. Frere, takes a small piece of thin glass, and with the writing diamond scratches a line corresponding to the piece of glass he wishes to remove, next a bradawl or other sharp instrument is placed in the centre of the space, the glass being laid upon a perfectly flat surface, such as thick plate glass.

A sharp tap upon the bradawl with a light hammer causes it to perforate the glass, but the cracks made in it do not extend beyond the line marked with the diamond. The fragments of glass are then carefully removed piecemeal with a pair of fine forceps, and the cell is complete. In many cases, however, the cracks do pass beyond the line, and thus the chance of removing the fragments from the centre is much diminished.

The method which I have been in the habit of employing for some years is this: I cement a square or circle of thin glass with marine glue to one of the circular or quadrangular rings of glass used for making deep glass cells, and alluded to in § 127: the hole in the centre being the exact size of that required to be made in the thin glass (Plate XXV, Fig. 101). When the marine glue is cold, a file is forced through the centre of the thin glass. The cracks thus produced do not run across that part of the glass cemented by the marine glue. The edges may then be filed square, and the thin glass only requires to be warmed in order to remove it from the cell. It may now be fixed upon the slide at once, or cleaned with potash and kept with others until required to be made into a cell.

In order to perforate the thin glass in making thin glass cells, Mr. Brooke takes two firm brass rings, ground perfectly flat, the diameter of one being a trifle less than that of the other. The piece of thin glass to be perforated is firmly pressed between them, and the writing diamond carried round so as to scratch each surface. The circular piece is then removed by a slight tap upon the surface on which the smallest circle has been scratched.

**126. Deeper Glass Cells.** — Supposing a cell a little deeper than any of the above is wanted we may proceed in a different manner (Plate XXV, Fig. 105):—a piece of plate glass of the proper thickness is to be cut with the diamond to correspond with the outside of the cell, next, from each side of this piece of glass, a strip of the required width is to be removed, and from its ends, corresponding strips are to be cut off. The central portion is taken away, and the strips thus cut out are *inverted* upon the slide upon which they are to be fixed with marine glue, care being taken to mark them in the first instance, so that they may correspond properly with each other. The marine glue is allowed to run well into all the corners. In this way a capital cell



is very easily and quickly made. Cells of various sizes and depths can be manufactured upon this principle. The surface of the glass rim should be ground upon the stone, and the superfluous glue removed in the ordinary manner.

**127. Small Deep Cells for Injections.**—By drilling a hole in a piece of plate glass, by cutting off sections of various thickness from thick glass tubing, or from thick square glass bottles, or from vessels moulded for the purpose,—excellent cells of various dimensions, and admirably adapted for mounting injections and other purposes, are made; but when the preparation is of considerable thickness, deeper cells than any of those to which I have alluded will be required. These may be made in glass, gutta percha, and some other substances. A *round or oval cavity* may be ground upon the surface of a piece of very thick plate glass. (Plate XXVI, Fig. 109.) Different forms of small deep glass cells are represented in Plate XXV, Figs. 100, 101, 103, 104. Moderately deep glass cells may be made also by grinding holes of the size required through thick plate glass. (Fig. 103.)

**128. Built Glass Cells** are those which are constructed by joining together, at the edges and ends, separate pieces of glass with marine glue or some other cement. The simplest form of built glass cell has been already described.

Good cells may be made from thick plate glass, the edges of which have been ground perfectly flat before they were united with the marine glue. Dr. Goadby used to make many of these cells, which can be formed upon this principle of very large dimensions. They may be obtained of Mr. Dennis, of St. John's Street Road, who has succeeded in making cells in this manner large enough to hold several quarts of fluid. Many cells of this description may be seen in the Hunterian Museum of the Royal College of Surgeons. They may be constructed as follows:—A strip of plate glass is cut off, of the proper height for the sides of the cell. From this, two pieces are to be cut off the desired length of the sides, and two pieces for the ends. The flat surfaces of these are to be cemented with marine glue, and all the edges ground perfectly flat together. (Plate XXVI, Fig. 108.) The ends are also to be very carefully ground square. They are then to be separated by heat and connected together at the corners in the proper position. (Plate XXV, Fig. 102.) When the four sides

Fig. 100.



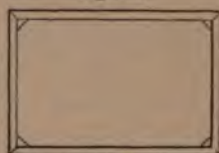
§ 127.

Fig. 101.



§ 125.

Fig. 102.



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Fig. 103.



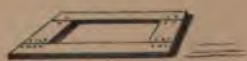
§ 127.

Fig. 104.



§ 127.

Fig. 105.



§ 126.

Fig. 100. Small cells for preserving injections and other thick preparations.

Fig. 101. To illustrate the manner in which the thin glass may be perforated for making thin glass cells.

Fig. 102. Shows the way in which the angles of a built glass cell are joined together.

Fig. 103. Glass cells made by grinding out the centre of a piece of plate glass.

Fig. 104. Large deep glass cells, for preserving opaque preparations.

Fig. 105. Illustrates a simple way of making a moderately thick glass cell.





have been thus joined together, one surface is to be carefully ground flat, and then cemented to the plate glass bottom. The other side, on which the cover is to be placed, may be ground flat afterwards. In order to increase the strength of these cells and to diminish the chance of leakage, it is well to cement small pieces of glass in the corners, and narrow stripes outside, where the sides are attached to the glass slab. (Plate XXVI, Fig. 107.)

These cells, of course, take some time to make, but they are exceedingly neat, and have but one serious drawback—a slight liability to leak, which is hardly to be wondered at when the number of the joinings is taken into consideration.

**129. Deep Glass Cells made by bending a strip of Glass in the blow-pipe flame.**—For some years past I have been in the habit of bending a long strip of glass in the blow-pipe flame, and cementing the extremities together in a similar manner whenever a cell of about half an inch in depth is wanted. The ordinary plate glass is very liable to crack as it becomes cool, but if *flatted flint glass* be employed the operation is simple enough. This glass, as well as the deep glass cells above referred to, may be obtained at Messrs. Powell's glass-works, Whitefriars. This cell has the disadvantage of not being perfectly clear. If flint glass could be flatted, ground, and polished like plate, it would be of much value to those who mount large objects in deep glass cells. (Plate XXVI, Fig. 110.)

**130. Moulded Glass Cells.**—Of late years moulded glass cells have been much employed for anatomical preparations, and the absence of joints renders them preferable to built glass cells. Large moulded cells are now made in Germany, the sides of which have been ground and polished, and thus a preparation can be seen within, almost as clearly as if the sides were composed of plate glass. These cells can be obtained for a much lower price than the built cells, and are, of course, not so liable to leak. They may be purchased at the glass-works, Whitefriars.

**131. Gutta Percha** may be moulded in a wooden case, and forms excellent cells where transparent sides are not required. I have several preparations which have been preserved for many years in large cells of this description. Gutta percha is most

useful for joining glass tubes to flat cells as may be required in forming cells for special purposes. (Plate XXVI, Fig. 106.)

**132. Troughs for Examining Zoophytes.**—These are deep but very narrow glass cells, the two surfaces consisting of very thin glass, so that the higher powers may be brought sufficiently close to the objects. The opening is above, so that the cell with living animals within may be placed upon the stage of the microscope, when the instrument is inclined, without any fluid escaping. It is convenient to have a glass partition in these troughs, by means of which objects may be placed in different parts of the cell. A convenient size is three inches long, an inch and a half deep, and a quarter of an inch in width.

**133. Animalcule Cage.**—Another very convenient form of cell is the one called animalcule cage. (See Plate XXXV, Fig. 157, Plate XL, Fig. 32). By means of its sliding cover a stratum of fluid of any required thickness can be obtained, and small living animals can be conveniently fixed in positions suitable for observation. For the examination of deposits in fluids this form of cell is also very convenient.

**134. Round Cells.**—My friend and colleague Dr. Guy has lately proposed a form of cell which possesses many advantages over those in common use. These are circular, and may be made of bone, metal, gutta serena, or glass, of various depths, and to suit transparent and opaque objects. Several forms have been made. They are all of the same external diameter, and are made to fit into a rim of equal size in a flat plate of wood, or metal, which can be placed in the field of the microscope. A small cabinet will contain many more preparations mounted in this manner than on the ordinary slips of glass. Dr. Guy has had some circular labels printed for these cells upon which the names of the preparations may be written, and as these are of different colours the various microscopic objects can be readily classified.

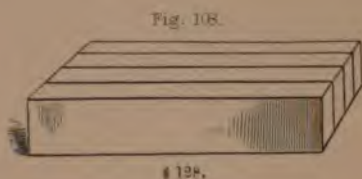
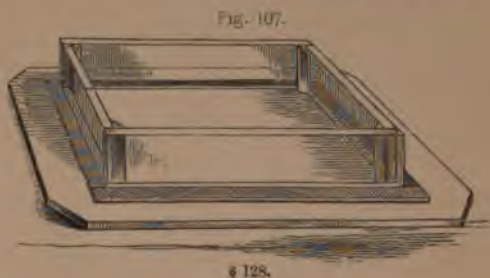


Fig. 106. To illustrate the manner in which cells of a peculiar shape may be made. The lower part is made of plate-glass, to which the tube is attached by gutta percha. This apparatus was made for examining the circulation in the branchia of a proteus. The smaller tubes were for the purpose of supplying the animal with fresh water.

Fig. 107. Large built glass cell.

Fig. 108. Shows the manner in which the sides of built glass cells are cemented together in order to be ground perfectly flat.

Fig. 109. Concave glass cell made by grinding out a cup-shaped cavity on the surface of a piece of very thick glass. It is afterwards polished.

Fig. 110. Deep glass cell, made by bending a piece of glass in the blowpipe flame.





## CHAPTER V.

ON EXAMINING OBJECTS IN THE MICROSCOPE. — *For Beginners only—How to Examine an Object in the Microscope.* GENERAL CONSIDERATIONS UPON THE STRUCTURE OF TISSUES. — *Flesh or Muscular Tissue—On Demonstrating the Anatomical Peculiarities of Tissues—The Anatomy of Organs more easily Demonstrated in the Lower Animals than in man and the Higher Animals—Of the Time after Death when Tissues should be Examined—Ciliary Motion.* OF PREPARING TISSUES FOR MICROSCOPICAL EXAMINATION. — *Of making Minute Dissections—Dissecting under the Surface of Fluid—Loaded Corks—Tablets upon which Dissections may be pinned out—Of obtaining Thin Sections of Different Textures for Microscopical Examination—Drying the Tissue before Cutting the Section—Hardening the Tissue—Horn—Hair—Making Thin Sections of Bone—Teeth—Sections of Wood—Of Dissecting Tissues under the Microscope with the Aid of the Compressorium—The Cell or Animalcule Cage.* OF THE IMPORTANCE OF EXAMINING OBJECTS IN VARIOUS WAYS. — *Appearance of the same Object in AIR, WATER, and CANADA BALSAM, by TRANSMITTED LIGHT, and under the influence of REFLECTED LIGHT and POLARIZED LIGHT.*

135. **For Beginners only. How to Examine an Object in the Microscope.**—Any one who purchases a microscope probably endeavours to look at some object through it as soon as it comes home, and of those who make such an attempt many fail completely, because they are not acquainted with the principles enunciated in this and the preceding chapters. The observer should go through the tables at the end of the volume ; but if too impatient and eager for action, he may proceed to work at once as follows :—

1. Place the microscope in the position represented in Fig. 47, Plate XV, the eye-piece and the low object-glass (the inch) being adapted to the microscope.

2. Take a dry bread crumb, about the size of a small pin's head, place it on a glass slide, and the slide upon the stage of the microscope.

3. Place an ordinary wax candle, or French, or other lamp in such a position that the upper surface of the crumb of bread may be lighted up, or use the bull's-eye condenser, so that a strong light is condensed upon the object, as in Fig. 47.

4. Screw down the body of the microscope until the object comes into focus and is seen distinctly.

The crumb of bread is examined as an *opaque object by reflected light*, and peculiarities of its surface are alone made out.

5. Alter the position of the light, if necessary, and so arrange the mirror that the light may be reflected from it, and caused to pass through the object (transmitted light), Fig. 48, Plate XV. Prevent the light from illuminating the surface as before. The object seems very dark and little definite is discovered.

6. Break the crumb up into several smaller pieces. This may be easily effected with the aid of a penknife. Most of them appear as angular particles. They seem dark because they are too thick for the light to pass through them, but here and there one appears more or less transparent.

7. One of the transparent pieces being in the field, remove the inch power and screw on the quarter of an inch object-glass, and examine the crumb. Still the appearance is not very definite or satisfactory, and little information is gained with regard to the structure of the crumb or of the nature of its component particles.

8. Next screw up the body of the microscope, and remove the slide from the stage. Carry a drop of water on the tip of one finger, and so cause the minute crumbs of bread to be wetted without their position being much altered, and carefully apply one of the pieces of thin covering glass (§ 84), after breathing upon the surface which is to come into contact with the fluid. The thin glass may be held in forceps or between the finger and thumb, and allowed to fall upon the wet crumbs very gradually by using a needle or a knife, as represented in Plate XXXVII, Fig. 174. Remove the superfluous moisture by the aid of the handkerchief, or with a piece of blotting paper, so that no water

will drop from the slide when it is placed upon the inclined stage of the microscope.

9. When the crumbs have soaked for a few seconds, give the thin glass two or three smart taps so as to crush them a little and make them spread out.

10. Bring the object as near the centre of the field as possible, and screw down the body of the instrument until the object comes into focus. Many new facts are now learned for the first time.

a. A number of small, oval, circular, angular and perfectly transparent particles are seen for the first time.

b. The dark indefinite appearance before observed is no longer visible.

c. Each transparent particle has a sharp and dark outline. Some are cracked, others exhibit irregularities of surface, while in some an indication of concentric lines may be observed. These bodies are starch granules or corpuscles of various sizes, modified by the heat of the oven. They appear clear and transparent now they are *examined in water*, instead of black and opaque as when they were examined before *in air*, because the refractive power of the water approaches more closely to that of the starch granule than the air. (See Plate XXXI, § 155.)

d. Probably some black spherical bodies or very wide and dark circular rings will be observed here and there. These are air bubbles. (Plate XXXVI, Fig. 161.)

11. Examine the thinnest possible shaving of deal wood or of a cedar pencil, and of mahogany or oak, a fragment of blotting paper, a piece of cotton and linen scraped as fine as possible, a small pinch of flour, ordinary starch, common pepper, cayenne pepper, powdered mustard, in the same way as the bread crumbs, taking care to allow them to soak in the drop of water for an hour or more, so that they may be perfectly wetted.

12. Subject pieces of moist tea leaves, very thin sections of potato and the peel of the potato, the skin or interior of an orange, lemon or other fruit, a piece of rhubarb, cabbage, or other vegetable, taking care that in all cases the pieces are *small enough*. They can easily be subdivided by a sharp penknife.

I strongly recommend the beginner to examine various specimens of jam and preserved fruits. As these vegetable tissues have long soaked in syrup, they have become exceedingly transparent, and admirably fitted for microscopical demonstration. The spiral vessels, woody and cellular tissues can be



obtained without any trouble, and the minute structure of the different vegetable tissues can be most clearly demonstrated.

The thinnest possible sections can be cut with a sharp thin knife (§ 75) from the firmest of these preserved fruits. The specimen may be placed in a little syrup for examination.

The action of syrup and glycerine will be discussed in Chapter X.

**136. The Structure of Tissues—General observations.**—The tissues of animals and plants for the most part are compound, and made up of several distinct elementary structures. For example, the smallest portion of flesh or muscular tissue, which can be removed with a knife or pair of scissors, is composed of several distinct structures. In the first place must be noticed the *proper substance* peculiar to muscular tissue, in which the characteristic contractile power resides. Secondly, at least in most cases, we find a tube composed of perfectly clear, *transparent, structureless membrane*, in which this contractile substance, or sarcous matter, is contained. Thirdly, there exists a certain quantity of *areolar tissue* (Figs. 112, 113, 115), which connects together these elementary fibres; and not unfrequently associated with this is a little *fatty or adipose tissue* (Figs. 118, 119). Fourthly, are *vessels* (Figs. 111, 114), lying between the elementary fibres just described, in which the blood circulates, for the supply of the tissue with its proper nutritive elements. In the fifth place we find nerve-fibres running in the same position as the vessels (Figs. 111, 114): and lastly, at least in relation with some of the fibres, are lymphatic vessels.

Thus, muscle is composed of several elementary structures, each having special anatomical peculiarities, and differing from the others in physical characters and chemical properties. Some of these structures refract light very highly; others, only in a very slight degree. One may be greatly altered or even destroyed within a very short time after the muscle has been removed from the body, or by the action of plain water, while others resist decomposition for a great length of time. The characters of one may be demonstrated when the muscle is examined in water; a second, when it is immersed in syrup or glycerine; a third, when the specimen is mounted in Canada balsam; while the arrangement of the delicate, transparent, capillary vessels cannot be satisfactorily made out unless a particular plan of preparation

Fig. III.



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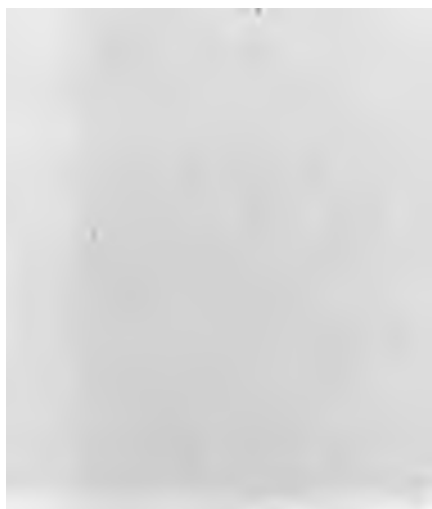
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§ 136.

Elementary muscular fibres from the diaphragm of the white mouse, showing the distribution of nerves and capillaries to striped muscle. Four fibres with their transverse markings. *a*, Sarcolemma. Nerve fibres given off from the bundle *b* in the upper part of the drawing. Capillary vessels. Masses of germinal matter ("nuclei") are seen in connection with the muscular fibres, with the nerves, and with the capillaries in all parts of the drawing.

[To face page 76.]





be adopted. Such is the nature of the textures in ordinary flesh which are demonstrable by the aid of the microscope.

In Plate XXVII a drawing of a beautiful specimen of elementary muscular fibre from the diaphragm of the white mouse is represented. This was prepared by injecting the vessels with glycerine and other substances in the first instance, as will be fully described in Chapter VII. The finest nerve fibres and capillaries are seen in this specimen, and the transverse striæ or markings on the elementary fibres are very distinct.

The chemist can detect a host of other compounds the presence of which the mere microscopist would ever remain unconscious of, for they are dissolved in the juices of the muscle, and therefore incapable of being detected by the eye alone.

The vast difference in the properties of the several textures above enumerated renders it very difficult to demonstrate all in one single specimen, for the circumstances which favour the exhibition of one structure will often render another quite invisible. Hence, before we can hope to demonstrate satisfactorily the anatomical peculiarities of any one of these different textures we must become acquainted with its general properties, and must consider the mode of examination likely to be most efficient in rendering these distinct.

The walls of the smallest vessels are so thin and transparent that it is necessary to fill them with some coloured fluid or material more or less opaque, if we wish to see the mode of arrangement of the vascular network (*see* Chapter VII, On Injection); while this same process, as ordinarily followed out, precludes the possibility of tracing the finer ramifications of the nerves, and other elementary tissues are hidden and compressed by the distended vessels. To demonstrate the nerves, all the other structures must be rendered as transparent as possible, by the application of a chemical agent, or by immersing the specimen in a highly refracting fluid (*see also* Chapter X). In order to show the membrane in which the *sarcous tissue* is contained, the latter must be ruptured within it in a perfectly fresh specimen, or it must be separated from it by pressure. By one plan of proceeding it may be shown that the elementary fibre of muscle may be divided longitudinally into a number of minute *fibrillæ*, arranged parallel to each other; while under other circumstances it can be separated transversely into a pile

of *small disks*, or into a number of small elementary particles of definite form and size, by the connexion of which to the contiguous particles, the *fibrilla*, or the *disks*, are produced, according as the particles adhere to each other most intimately by their sides or by their extremities. I might adduce many other instances of the necessity of studying the general character of tissues before any minute examination of the individual structures is attempted, but this is sufficient.

**137. On Demonstrating the Anatomical Peculiarities of Tissues.**—Now, some observers who have not sufficiently considered the different characters of the elementary structures of which most of the organs of the body are composed, have strongly objected to what they term *methods of preparation*, asserting that by these processes, structures are even *formed* which have no real existence in the natural state of the part. For this view there is some reason. Doubtless, from the examination of a dead tissue we can form but an imperfect conception of the beauty of its elementary parts, and their wonderful adaptation to the office they are designed to perform in the animal economy; neither can we form an idea of the changes it undergoes during life; and we must remember that there is no known fluid in which we can immerse a specimen for examination, which possesses the precise characters of that which bathes the tissue during its lifetime. Serum may, perhaps, be the nearest approach to such a fluid, but there is every reason to believe that this differs from the fluid surrounding the primitive particles almost as much as some artificial media which have been proved by experience to give very satisfactory results. Objectors to the preparation of tissues have not satisfactorily proved that many of the structures which we see after death have a precisely similar appearance during life, and it is more than probable that many of the more delicate tissues have never been seen by any one in the condition in which they exist during life. I believe that the amount of opacity which is absolutely necessary for seeing some of these is quite inconsistent with their natural condition, and is the result of a change which has never been fully appreciated, though, perhaps, some idea of its nature may be formed by considering the characters of fibrin in the circulating blood, and fibrin removed from the organism and coagulated, or those of

PLATE XXVIII.

Fig. 135.



§ 136.

Fig. 125.



§ 136.

Fig. 112.



§ 137.

Fig. 116.

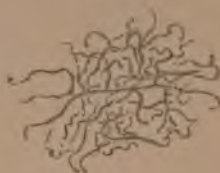


Fig. 117.



Fig. 118.



§ 137.

Fig. 113.



§§ 136, 138.

× 700

Fig. 112. Yellow fibrous tissue from the ligament of the neck of a sheep. Fig. 113. White fibrous tissue from tendon. Fig. 114. Muscular fibre, with nerve fibres and capillary vessels. Fig. 115. Fibres of yellow elastic tissue. Fig. 116. Capillary with nerve fibres around it. From the frog. Fig. 117. Tracheae from an insect. Fig. 118. Adipose tissue. Fat vesicles, with germinal matter or 'nuclei.'

[To face page 78.]





albumen dissolved in the serum, coagulated but transparent in many of the tissues, coagulated and opaque after the addition of different reagents.

From what I have just observed it must be evident, that the clear demonstration of the structure of any individual organ of the body is a somewhat difficult matter, and requires a considerable amount of knowledge of the chemical and physical characters of the tissues, as well as patient investigation and earnest study, which will alone enable us to make artificially a fluid which shall possess the most important characters of that which surrounds the tissue during life.

**138. The Anatomy of Organs more easily Demonstrated in the Lower Animals than in Man and the Higher Animals.**—In consequence of the great complexity of the structure of many of the tissues of the higher animals their rapid change after their removal from the body, and their extreme delicacy, anatomists have long been in the habit of resorting to the examination of textures in the lower forms of animal life for obtaining an insight into the structure of parallel tissues in the higher, and with considerable success. I can adduce no better example of the great value of such an appeal to the simpler forms of animal life than occurs in the case of the *kidney*.

In animals generally, this gland consists essentially of a vast number of long and highly tortuous tubes—which in the higher members of the class are packed so closely together that they form a firm and very compact organ, the general characters of which are familiar to all—and of vessels bearing a particular relation to these tubes. In such a kidney it is impossible, under ordinary circumstances, to follow a tube for any very great length, as the observer will be convinced if he looks at a specimen in the microscope; but in the lower animals the kidney is less compact, and the several tubes are not so intimately connected together. Indeed, in many of them the kidney is prolonged into a thin, transparent, almost thread-like organ, which extends into the thoracic portion of the animal. In this situation in the common *newt* or *eft* (Triton or Lissotriton) we have, so to say, a natural dissection of the elements of the gland structure, and we may *demonstrate* an arrangement, the existence of which we can only *infer* by an examination of thin sections of the compact kidney of mammalian animals. Single tubes, with the structures con-

nected with them, may be traced throughout their entire length, and are quite separate from one another. I need hardly observe, that it would be vain to attempt to make such a dissection artificially.

Many other instances of the value of this kind of investigation might be adduced of equal interest and importance, but instead of occupying time in this manner I will most strongly urge upon all those who are likely to prosecute researches upon the characters of any particular tissue or organ, the importance of investigating carefully its nature in the different members of the creation, and especially in the lowest forms in which its existence has been proved,—for there we may be sure to find it in its simplest condition, and the mind will be better able to appreciate the exact meaning of the structures which are superadded, and the more elaborate anatomical detail which is met with in the higher animals, than if we commenced our researches upon the most perfect examples of the structure.

**139. Of the Time after Death when Tissues should be Examined.**—I must also make a few remarks upon the time when the examination may be carried on with the best chance of success. Some animal tissues require to be examined very soon after death, or their characteristic peculiarities are lost. Soon after death a shrinking or collapse of the soft germinal matter (the so-called 'nucleus') occurs, and this alteration has led to the view that the nuclei lie embedded in spaces or vacuoles in the tissues. During life, and especially in the early and more active period of growth, the matter of which the nucleus is composed is *continuous with the tissue*.

**140. Ciliary Movement.**—Upon certain surfaces in the higher animals, and to a greater extent in the lower classes, we find that the cells which generally form the outer coating to more delicate structures beneath, are provided with very active vibratile processes, or *cilia*, which by their movement create currents often of some considerable power. These movements are sometimes required to promote the rapid removal of foreign bodies which would injure delicate surfaces if they came in absolute contact with them, or for promoting a constant change in the fluid medium by which the animal is surrounded. The importance of cilia in effecting the latter object is seen in the

Fig. 119.



§ 137.

Fig. 120.



§ 137.

Fig. 121.



§ 141.

Fig. 123.



§ 138.

see this .....  $\times 403$   
 see this .....  $\times 130$

Fig. 119. Fatty tissue showing fat vesicles, the crystalline fat has separated from the oily fat.  
 Fig. 120. Small vessel dividing into capillaries.  
 Fig. 121. Arrangement for making minute dissections.  
 Fig. 123. Newt dissected to display the thin part of the kidneys, under which a needle, *a*, has been placed. In this part of the kidney, ciliary motion is seen very readily.

[To face page 80.]





greater number of shell fish, which are stationary throughout life, and are not provided with an apparatus for promoting a continual change of the fluid which bathes the surface of their respiratory organs. In these animals this great object is entirely effected by the agency of these small cilia.

*Ciliary Motion* endures for a longer or shorter period after death, and is entirely independent of the nervous system. In the active birds it ceases very soon, but in the more slowly-nourished, cold-blooded animals it often lasts for many days after death.

Ciliated epithelium can always be seen in the mucus scraped from the tongue of the frog, in the kidneys of this animal and of the newt, and on the gills of the mussel and oyster, of which a small piece may be removed with a pair of scissors. It is important to moisten ciliated epithelium with a little serum of the animal, as water soon puts a stop to the movements. The movements are excited by the action of very dilute potash.

#### ON PREPARING TISSUES FOR MICROSCOPICAL EXAMINATION.

**141. Of Making Minute Dissections.**—Minute dissections are usually carried on under the surface of fluid with the aid of small scissors, needles or small knives, and forceps. If the preparation has been preserved in spirit or other solution, it must be dissected in the same fluid, but in ordinary cases clear water may be used. The microscopist should be provided with a few small dishes, varying in size, and about an inch or more in depth. The large built cells make very good troughs for dissecting in, but small circular vessels are made on purpose.

**142. Loaded Corks.**—The object to be dissected is attached to a loaded cork by small pins (Plate XXX, Fig. 124). We may take a piece of flat cork rather smaller than the cell, and then cut a piece of sheet lead somewhat larger than the cork. The edges of the lead are then folded over the cork and beaten down slightly with a hammer, and may afterwards be filed with a rough file.

The object being fixed upon the cork and placed in the cell, fluid is poured in until it just covers the surface (Plate XXIX, Fig. 121). A strong light is then condensed upon it by means of a large bull's eye condenser, or by a large globe full of water.



With a strong light, magnifying glasses are not required ; and I have always found that delicate dissections could be made with the greatest facility without the aid of a dissecting microscope, provided a strong light was condensed upon the object. Occasional examination of the dissection with a lens of low power is advantageous ; but if a lens be employed during the dissection there is great danger of accidentally injuring the specimen, as it is impossible to judge of the distance which the needle point may be beneath the surface of the fluid. Minute branches of nerves or vessels may in this way be followed out, and small pieces of the different tissues into which they can be traced may be removed for microscopical examination with a pair of fine scissors. Membranes may be dissected from the structures upon which they lie in a similar manner. By this plan the nervous system of the smallest insects can be very readily dissected. The mode of proceeding is represented in Fig. 121.

**143. Tablets upon which Dissections may be Pinned out.**—Many preparations require to be arranged in a particular position previous to being mounted as permanent objects. *Slabs of wax* are usually employed by anatomists for this purpose, but when transparency is required the dissections may be attached by threads to thin plates of *mica*.

I have found that the best slabs may be made of a mixture of *wax* and *gutta percha*, in the proportion of one part of the former to two of the latter. The ingredients are to be melted in an iron pot, over a clear fire, and well stirred. When quite fluid, the mass may be poured upon a flat slab and allowed to cool. Thin cakes of about the eighth of an inch in thickness are thus obtained, and they can easily be cut with a knife to fit the cells intended for the preparation. Pins or small pieces of silver wire may be inserted into these slabs, and will adhere firmly although the slabs are very thin.

**144. Of obtaining Thin Sections of different Textures for Microscopical Examination.**—The instruments required for obtaining thin sections of soft tissues have been described in Chapter III.

It is scarcely necessary to observe that such different textures as muscular fibre and gland structures, and other soft tissues, require a process for cutting them different to that which is

applicable for cutting thin slices of such tissues as hair, horn, bone, or teeth.

Where thin sections of no very great extent of tissue are required they may be obtained by scissors (§ 73), by the ordinary scalpel (§ 74), by the double-edged knife (§ 75), or by Valentin's knife (§ 77). Whenever a thin section of a tissue is made, the instrument employed must be thoroughly wetted with water, and the section, after its removal, should be carefully washed, by agitating it in water, or by directing a stream of water upon it from the wash-bottle (§ 180, Fig. 173, Plate XXXVII). This washing is absolutely necessary to remove from the surface of the section particles of *débris*, which would render the appearances indistinct, and interfere with the clearness of the specimen when it was subjected to examination in the microscope. The section may then be transferred to the fluid in which it is to be examined or preserved.

**145. Drying the Tissue before Cutting the Section.**—There are, however, many tissues, of which sections cannot be obtained in this simple manner,—thus it is almost impossible to cut sections of soft membranous textures perpendicular to the surface, sufficiently thin for examination. In such cases, it is advisable to pin the texture out upon a board when perfectly fresh, and expose it to the atmosphere until quite dry. Thin sections may then be cut very easily, and upon being moistened with water will resume their recent appearance. The very delicate nervous tissue of the retina may be cut into very thin sections by drying the eye which has been cut open, so that it may be pinned out flat on a board. The vitreous humour is not to be entirely removed, as it protects the retina and dries up with it. Very thin sections of skin and many other tissues may be obtained by this process.

**146. Hardening the Tissue.**—Other textures, again, require special treatment in order to render them sufficiently hard to enable us to cut thin sections. Some require boiling for this purpose, others soaking in alcohol, or chromic acid, or in syrup, while not a few require special modes of treatment, which are applicable to them alone.

**147. Horn.**—Thin sections of horn and textures of this des-

cription may be cut with a sharp strong knife (Plate XXIII, Fig. 89).

**148. Hair.**—There are many ways of obtaining thin transverse sections of hair. Thus a number of hairs, may be united together by a little gum, so as to form, when dry, a firm hard mass. Thin sections of this can readily be made, with a sharp knife, and the individual pieces may be separated from each other, by the application of a drop of water. These may be mounted in fluid, or dried and preserved in Canada balsam (§165).

Or the hairs may be placed between two pieces of cardboard, or between two flat pieces of cork, and when tightly pressed in a vice, thin sections of the hair, including the cardboard and cork, can be obtained with a sharp knife. For cutting thin transverse sections of hair, my friend Professor Weber of Leipzig, recommends a very simple expedient. He suggests that the beard should be shaved very closely, and then after a few hours shaved again. In this way excessively thin sections of hair in great numbers may be obtained.

**149. Making Thin Sections of Dry Bone.**—For obtaining thin sections of bone, a totally different process is requisite. In the first place, a section as thin as possible is removed from the bone with the aid of a thin sharp saw (Plate XXIII, Fig. 90). This may be made somewhat thinner by a file, and afterwards ground down to the required degree of tenuity upon a hone. The best stones for this purpose are the Arkansas oil stones or the Turkey stones, which have been ground perfectly flat. The section may be kept in contact with the stone by the pressure of the thumb, or with a piece of cork, or by the finger; or lastly, it may be rubbed between two hones, a proceeding which saves much time.

It is to be ground down with the aid of a little water, and when sufficiently thin it may be subjected to examination in the microscope. It will, however, be found, that the beauty of the tissue is completely obscured, owing to the number of scratches upon its surface. These may be removed by rubbing the section upon a dry hone, and afterwards upon a piece of plate glass. After the piece of bone has been properly polished, no lines will be seen upon it, when it is examined in the microscope.

**150. Teeth.**—Sections of dry teeth cannot be advantageously



PLATE XXX.

Fig. 124.



§ 142.

Fig. 126.



§ 152.

Fig. 125.



§ 153.

Fig. 127.



§ 153.

Fig. 124. Loaded cork, upon which objects for dissection may be pinned out.  
 Fig. 125. Instrument for cutting thin sections of wood, &c.  
 Figs. 126, 127. Different kinds of compressorium, for pressing or tearing-up tissues under the microscope.

[To face page 84.]





prepared in this manner, owing to the very brittle nature of the enamel. The better way is to grind the tooth down at a dentist's lathe until a section sufficiently thin be obtained.

Sections of *fresh* bone and teeth may be prepared moist so as to show many more important points in their structure and mode of growth, according to the plan described in Chapter X. After they have been soaked for some time in glycerine and acetic acid, very thin shavings even of enamel may be obtained with a strong sharp knife. The calcareous matter may be dissolved out from specimens prepared by carmine, and thin sections easily made of the modified matrix.

151. Sections of shells of many of the lower animals, and the hard shells and stones of fruit may be made in a similar manner.

152. Sections of Wood may be made with the aid of a little instrument figured in Plate XXX, Fig. 125. A piece of wood, after having been allowed to soak for some time in water, is placed in the hole, and kept in its position by the side screw. Upon turning the lower screw the wood is forced above the brass plate. A clean section is now made with a sharp strong knife or razor. By turning the screw beneath, very slightly, the wood is forced above the surface of the brass plate, and thus a section of any required thickness may be obtained.

153. Dissecting Tissues under the Microscope with the aid of the Compressorium.—In many cases the observer wishes to dissect an extremely delicate structure *under the microscope*, for in this way much information can often be acquired with reference to the exact relation existing between the structural elements of the tissue. This object may be gained by means of a little instrument termed a *compressorium*, which consists simply of a convenient arrangement by which pressure can be applied to an object while under examination (Plate XXX, Figs. 126, 127). This pressure being applied gradually, the texture becomes frayed out as it were, and particular structures can often be teased out from a tissue, and demonstrated more distinctly than by any other method.

The structure of the compressorium is very simple. Many different forms have been recommended, one of the simplest consists of a thick brass plate with a hole in the centre to admit the light. On one side of this is situated the fulcrum of a lever, the

short end of which acts upon a circular ring carrying the thin glass to cover the preparation, while to the longer arm is attached a screw, which by being turned causes the thin glass to be pressed tightly upon the object placed upon a piece of plate glass situated upon the plate of the compressorium. A more perfect form of instrument has been arranged by Mr. Highley. It is represented in Plate XXX, Fig. 126.

The plate glass is usually fixed in the hole in the brass plate, but it is more convenient to have a ledge attached to one side, so that an ordinary plate glass slide may rest upon it. With such an arrangement, the tissue to be examined can be placed as may be thought desirable, upon any part of the glass before it is removed to the compressorium.

A very convenient form is employed by M. Quatrefages, in which it is possible to examine the object upon either side.

154. The Cell or Animalcule Cage (Plate XXXV, Fig. 157) also serves the purpose of a compressorium when a very great amount of pressure is not required. It is important that the shoulder upon which the cover fits should be at least as wide as the one figured, otherwise when the glasses are not cleaned immediately after use, solutions which have been examined are apt to dry and prevent the removal of the cover without much trouble.

#### OF THE IMPORTANCE OF EXAMINING THE SAME OBJECTS IN VARIOUS WAYS.

Many objects require examination in several distinct ways before an accurate idea of their general structure can be obtained. It is in many instances of the utmost importance to examine an object by *reflected light* as well as by *transmitted light*, and to observe the peculiarities of structure when it is surrounded with *air*, or immersed in *water*, or in a highly refracting fluid, such as *glycerine*, *oil*, *turpentine*, or *Canada balsam*. Not less valuable is the information we derive from the application of certain *chemical reagents* (see Chapter VIII). The microscopical observer must bear in mind that in order to make out the exact nature of any texture it is necessary to subject it to various different processes of observation, and to the action of certain chemical reagents, according to the *transparency or opacity*, *density*, *refractive power*,

and *chemical composition* of the specimen. So also he must submit the object to examination with *high powers* and *low powers*.

**155. Appearance of the Same Object examined in Air, Water, and Canada Balsam, by Transmitted Light, and under the influence of Reflected Light and Polarized Light.**—In Plate XXXI specimens of the same structure (spherical crystals of carbonate of lime and octohedra of oxalate of lime) magnified in the same degree, are represented.

*In Air.*—In Fig. 128 crystals of carbonate of lime, and in Fig. 134 octohedra of oxalate of lime are shown by *transmitted light* in air mounted in the dry way, and it will be noticed how very dark and thick the outer part appears, and how impossible it is to make out the ultimate arrangement of the former crystals.

*In Water.*—In Figs. 129 and 135 the same crystals are seen in water. The outer part is still very dark and thick, but in the carbonate of lime a few lines may be observed radiating from the centre of the crystals towards their circumference, although not very distinctly.

*In Canada Balsam.*—In Figs. 130 and 136 the crystals are shown immersed in Canada balsam. Here the outline appears as a sharp well-defined line. In the case of the carbonate of lime a vast number of narrow lines are seen radiating from the centre of the crystal towards its circumference, which shows that it is really made up of a congeries of minute acicular crystals.

*By Reflected Light.*—In Figs. 131 and 133 the crystals are represented as they appear when examined by reflected light. The globular form, and yellowish colour of the carbonate of lime, are very distinctly seen, and the surfaces of the crystals generally seem slightly rough, while some appear to be covered by minute elevations.

*By Polarized Light.*—In Fig. 132 another preparation of the crystals of carbonate of lime is seen under the influence of polarized light. Each crystal exhibits a black cross which alters its position and appearance as the *analyzer* (§ 34) is rotated. These important points might be illustrated by a vast number of other substances. I cannot too strongly advise the observer to subject various microscopical structures to examination in *air*, *water*, and *Canada balsam*, and by *direct* or *reflected*, as well as under the influence of *transmitted light*, and in some cases by *polarized light*.





Fig. 128.



Fig. 129.



§ 155.

Fig. 130.



Fig. 131.



Fig. 132.



§ 155.

Fig. 133.



Fig. 134.



Fig. 135.



§ 155.

Fig. 136.



Fig. 137.



§ 243.

Fig. 128. Spherical crystals of carbonate of lime, examined by transmitted light in air. Fig. 129. The same in water. Fig. 130. The same in Canada balsam. Fig. 131. The same under the influence of reflected light. Fig. 132. The same under the influence of polarized light. Fig. 133. Octohedral crystals as seen by reflected light. Fig. 134. The same in air by transmitted light. Fig. 135. In water. Fig. 136. In Canada balsam. The thin lines in the last case are caused by the refractive power of the crystal and that of the medium in which it is immersed being nearly equal. Fig. 137. To illustrate the appearance of crystals when examined in their own mother-liquor.

[To face page 88.]





## CHAPTER VI.

ON THE EXAMINATION OF TISSUES AND THEIR PRESERVATION AS PERMANENT OBJECTS.—*General Considerations with reference to the Nature of the Medium in which Tissues should be placed for Examination.* EXAMINATION AND PRESERVATION OF STRUCTURES IN AIR. EXAMINATION AND PRESERVATION OF SUBSTANCES IN AQUEOUS FLUIDS. EXAMINATION AND PRESERVATION OF SOFT TISSUES.—*Muscle—Villi, Adipose Tissue—Nerve Fibres—Arrangement for pressing down the Thin Glass Cover upon the Preparation while the Brunswick Black is drying—Examination of Vegetable Tissues—Of the Circulation in the Cells of Vallisneria—Anacharis, Anchusa, &c.* EXAMINATION AND PRESERVATION OF OBJECTS IN CANADA BALSAM.—*Examination of Hard Tissues: Bone.* AIR-BUBBLES. OIL-GLOBULES.—*Blood-globules—Fungi.* OF THE SEPARATION OF DEPOSITS FROM FLUIDS.—*Conical Glasses—Pipettes—Removing the Deposit with the Pipette—Separation of Deposit when very Small in Quantity—Examination of Infusoria—Vorticellæ—Zoophytes—On separating the Coarse from the Finer Particles of a Deposit—Method of obtaining the Silicious Skeletons of Lower Organisms—Wash-bottle—Of keeping Preparations in the Cabinet.*

OF THE EXAMINATION OF TISSUES AND OF THEIR PRESERVATION  
AS PERMANENT OBJECTS.

Microscopic objects require to be mounted in different media according to the nature of the texture and the particular tissue which it is intended to display.

156. General Considerations with reference to the Nature of the Medium in which Tissues should be placed for Examination.—If the structure be dry and very thin, or if it is required

reflected light only, but very thin dry tissues, like the epidermis from different parts of plants, may be examined by reflected or by transmitted light.

#### EXAMINATION AND PRESERVATION OF SUBSTANCES IN AQUEOUS FLUIDS.

**158. Examination of Substances in Fluids.**—I have already drawn attention to the most important points to be borne in mind, with reference to the examination of substances in aqueous fluids (§ 155). In choosing a fluid in which the specimen is to be immersed, its chemical composition, its transparency, and its refractive power must be considered. The different preservative solutions described in Chapter III, page 58, may be used for the preservation of a variety of objects in fluid. If we wish for a fluid closely resembling water, but possessing the property of preserving the specimen, we may use the *solution of naphtha and creosote* (§ 102), or *naphtha and water*, or carbolic acid and water. If we require a fluid of higher specific gravity, some of the saline solutions, diluted with a proper quantity of water, may be used. If we wish for a solution which refracts highly, we may employ glycerine, or a mixture of glycerine and gelatine; while, if we require a fluid which has the property of hardening the structure, we may immerse it in a solution of *chromic acid*, *bichromate of potash*, *corrosive sublimate*, or *diluted alcohol*.

In all cases the substance should be immersed for some time in the fluid, in which it is to be preserved, before being mounted permanently. The cell made of *Brunswick black* or the thin glass cell, or other forms which I described in Chapter III, may be chosen according to the dimensions of the specimen. The object and fluid being placed in the cell, the thin glass cover is applied, with the precautions to which I shall presently advert. The superfluous fluid is removed with a piece of blotting paper, or a soft cloth, and after the edges have been allowed to dry a little, they are anointed with a thin layer of *Brunswick black*.

Almost every organized structure, and especially the soft moist tissues of the bodies of animals, may be advantageously preserved in fluid. The solution which is employed for preserving a structure should resemble as nearly as possible in density and refractive power, the fluid which bathed it during life.

**159. Examination and Preservation of a Soft Tissue; Muscle.**—Suppose a portion of muscular fibre is to be examined under the microscope. A small piece may be removed with a pair of very fine scissors, and placed carefully upon the glass slide. With the aid of two needles it may be torn into very small shreds, and it is then to be moistened with a little water dropped upon it from the finger, or from a pipette, or from the wash-bottle; or instead of water, a drop of serum, of syrup, or of glycerine may be added to it, but in this case it should be allowed to remain in the syrup or glycerine for some time, so that it may be thoroughly permeated by the more dense solution. Next a square or circular piece of thin glass held in a pair of fine forceps is gently breathed upon and applied to the surface of the liquid, being brought into contact with it, first on one side, and then allowed to fall down very gradually with the aid of a needle or piece of fine wire placed underneath one edge, until it is completely wetted (Plate XXXVII, Fig. 174). Lastly, any superfluous fluid is to be absorbed by a cloth, or a small piece of fine sponge or blotting paper, and the slide placed in the field of the microscope for examination.

It is important to prevent the entrance of air bubbles (Plate XXXVI, Fig. 161) during the application of the thin glass cover, and if any are visible in the tissue or surrounding fluid, before it is applied, it will be better to wait a few minutes until they rise to the surface of the liquid and burst, before allowing the thin glass cover to fall in its place. While time is allowed for this to take place the specimen should be covered with a small glass shade to prevent dust falling upon it (Plate XXIII, Fig. 92, § 85).

It is advisable not to remove too much of the fluid, for fear the thin glass should press so heavily upon the preparation, as to cause the several structures of which it is composed to be squeezed together and the specimen rendered confused. The observer will find it very useful to place a piece of hair or hog's bristle, between the thin glass and the glass slide, by which means too great pressure will effectually be prevented. The same effect is obtained by using a glass cell, but it will be found, I think, that it is more convenient to pursue the plan just described in the mere *examination* of most tissues than to place them in a glass or other cell.

Sometimes it is desirable to examine specimens while warm. This may be effected by allowing a current of heated air to ascend through a copper tube, a piece of glass being placed at the



Fig. 133.



§ 160.

Fig. 140.

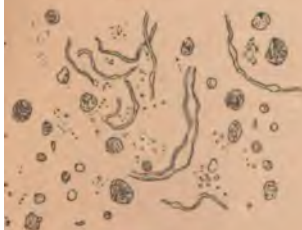


§ 160.

Fig. 141.

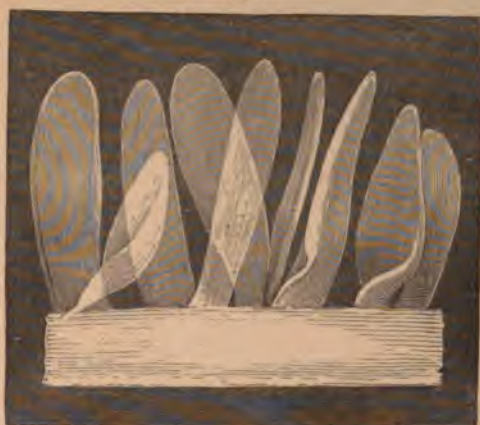


Fig. 144.



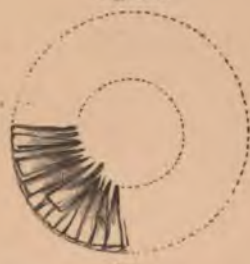
§ 160.

Fig. 139.



§ 160.

Fig. 142.



§ 160.

Fig. 143.



§ 160.

Fig. 145.



× 130

Fig. 138. Villi and follicles. Todd and Bowman. Fig. 139. Large flat villi of bird. Todd and Bowman. Fig. 140. Adipose tissue, with areolar tissue. Fig. 141. Arrangement of epithelium in a follicle. Fig. 142. Arrangement of epithelium round a villus. Fig. 143. Epithelium of a villus. Fig. 144. Nerve fibres altered by water. Fig. 145. E. from the follicles of the skin.

[To face 7]



lower part of that portion of the instrument which lies upon the stage. A circular hole is made in the upper surface, and over this the glass slide is placed. The arrangement will be at once understood by reference to the drawing (Plate XXXV, Fig. 158).

Whenever a specimen is to be preserved permanently in fluid, it should be immersed in the solution in which it is intended to remain for several hours previous to being mounted, so that it may be thoroughly saturated with it in every part. The fluid may be placed in a moderately deep cell, in a watch-glass, or in a cup of one of the palettes used by artists, from which it may afterwards be removed to the slide. The thin glass having been applied, and all superfluous fluid removed, a thin layer of Brunswick black is to be carefully placed round the edge so as to cement the thin glass to the slide. When this is dry other layers are to be applied successively until the joint is considered quite tight. The cement adheres better to the glass-slide if it is roughened previously by grinding in this part, or it may be scratched with the writing diamond just where the cement is to be placed. All objects, except the very thinnest, if preserved permanently in fluid should be placed in a cell, because there is a much better prospect of their being kept permanently, than when placed upon the glass slide in the manner employed for examining the specimen temporarily. The chance of air getting into the cell is much diminished if the cement which is used possesses slight elastic power, so as to admit the alteration which necessarily takes place in the volume of the fluid under variations of temperature. Mr. Brooke always adds a few drops of a solution of India-rubber to the Brunswick black, and this admirably fulfils the end in view.

**160. Examination of Villi, Adipose Tissue. Nerve Fibres.**—Several animal tissues are represented in Plate XXXII, Figs. 138 to 145. One of the best plans to demonstrate the villi, which project from the surface of the mucous membrane of the intestine is the following:—A stream of water is allowed to flow over the surface so as to cause the villi to fall in one direction. A clean cut is then made across the intestine, and the villi caused to fall in an opposite direction by the stream of water. When a very thin section is removed from the freshly cut surface, one or two rows of entire villi will be readily obtained. The epithelium is often removed by this process. Its arrangement is represented

Fig. 138.



§ 160.

Fig. 140.

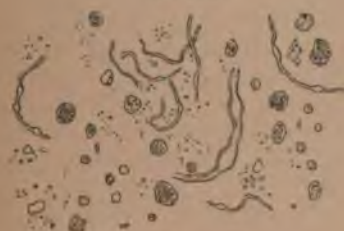


§ 160.

Fig. 141.



Fig. 144.



§ 160.

Fig. 139.



§ 160.

Fig. 142.



§ 160.

Fig. 143.



§ 160.

Fig. 145.



×130.

Fig. 138. Vili and follicles. Todd and Bowman. Fig. 139. Large flat vili of bird. Todd and Bowman. Fig. 140. Adipose tissue, with areolar tissue. Fig. 141. Arrangement of epithelium in a follicle. Fig. 142. Arrangement of epithelium round a villus. Fig. 143. Epithelium of a villus. Fig. 144. Nerve fibres altered by water. Fig. 145. Endites from the follicles of the skin.

[To face page 94.]



in Fig. 142, and in Fig. 143 some of the separate cells are seen.

A portion of adipose tissue, with the white and yellow elements of areolar tissue, is represented in Fig. 140.

*Nerve Fibres*, when examined by the usual methods, and especially when placed in water, become much broken up. It seems that the water alters the white substance of Schwann, which consists of soft and viscid matter, containing a large quantity of a peculiar form of fatty matter. Nerve fibres altered by water are represented in Fig. 144.

**161. Arrangement for Pressing Down the Thin Glass Cover upon the Preparation while the Brunswick Black is Drying.**—There are some substances which require slight pressure to display their peculiarities, and it is necessary to be provided with an arrangement for keeping down the glass cover until the cement which is to fix it in its place is dry. A very simple way of effecting this is, to place a small piece of wood, about an inch in height, upon the cover. This may be fixed in its place by passing a piece of thread over it, and tying it at the back of the slide; or the wood may be kept in its place by a vulcanized India-rubber ring. My friend Mr. White has devised a very simple and ingenious apparatus for this purpose. It consists of a bent lever, which, by acting upon a screw, can be forced down upon the thin glass with the amount of pressure required. Another form of instrument, designed by the Rev. G. Isbell, is seen in Plate XXXV, Fig. 159. The slide must be allowed to remain until the varnish be thoroughly dry. The compressorium may also be employed for the same purpose, by inserting a small piece of cork between the thin glass to which the pressure is to be applied, and the glass of the compressorium itself.

Mr. Hoblyn, of Bath (*"Archives of Medicine,"* Vol. III, p. 140), has invented an ingenious arrangement for the same purpose. In this instrument, a number of slides may be placed at the same time, and a graduated pressure exerted upon them. (See Plate XXXVII, Fig. 176.)

**162. Examination of Vegetable Tissues.**—The examination of vegetable tissues is conducted upon the same general principles as that of animal textures. The spiral vessels of plants can in many instances be obtained by boiling the stem of the plant for



some time in water. Those of rhubarb are very large, and may be selected for examination. Many plants exhibit circulation in the cells of which they are composed, and are very favorable microscopic objects.

143. Examination of the Circulation in the Cells of *Vallisneria*.—Suppose we wish to examine the circulation in the cells of the thin leaf of the *vallisneria spiralis*, we may proceed as follows:—A small portion is cut off from the plant, and a very thin slice removed from the surface with a sharp thin knife, so that the cells within the leaf may be brought clearly into view. The manipulation to which the piece is thus necessarily subjected, has the effect of retarding or even of stopping the circulation for a time. If, however, the section be kept for a short time in water it soon recommences. It is a good plan when we wish to exhibit specimens of this beautiful plant, to cut several sections of the required size, and place them in a small bottle of water in a warm room or in the pocket, for an hour or more before they are submitted to microscopical examination.

In Plate XXXIV, Fig. 147, a branch of *Anacharis alismastrum* is represented. It consists of long slender stems which bear a series of three narrow leaves of a pale green colour at intervals of about a quarter of an inch apart. The leaves when full grown seldom exceed a length of three-eighths of an inch. Fig. 148 shows the irregular shape and position of the cells in one of the leaves of this plant. The thickness of the central part of the leaf is composed of two layers of such cells, but at the margin only one layer exists. Fig. 149 represents one of the hollow spines or hairs at the margin of the leaf of the *anacharis*. It appears that when the circulating corpuscles arrive near the apex of the spine where the cell wall is indurated, as shown by a brown discoloration, they do not pass quite to the apex, but are invariably hurried across the cell, as seen at *b* in the figure. The three drawings above referred to have been taken from Mr. Wenham's paper "On the circulation in the leaf cells of *Anacharis alismastrum*." (*"Mic. Journal,"* Vol. III, p. 281.)

In Plate XXXIV, Fig. 150, is represented a hair or spine from the stalk of *Anchusa paniculata*, one of the *Boraginæ*. This is also taken from a drawing by Mr. Wenham (*"Mic. Journal,"* Vol. III, 49). The mode of growth and circulation of the sap-corpuscles are well shown. These accumulate and gradually



Fig. 185.



§ 164

Fig. 187.



§ 163.

Fig. 148.



§ 163.

Fig. 149.



§ 163.

Fig. 146. Small vivaria and fern case. Fig. 147. A branch of *Anacharis aleinastrum*.

Fig. 148. Cells of *Anacharis*, after Mr. Wenham. Fig. 149. Cells and hollow space of *Anacharis*, after Mr. Wenham.



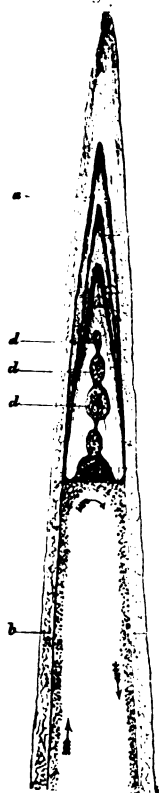
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PLATE XXXIV.

Fig. 150.



§ 163.

Fig. 151.



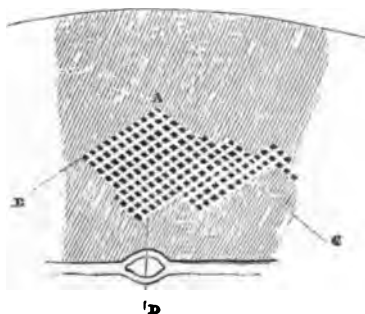
§ 163.

Fig. 152.



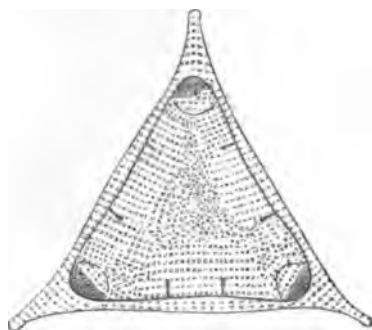
§ 171.

Fig. 152.



§ 174.

Fig. 153.



§ 171.

Fig. 154.



§ 174.

Fig. 155.

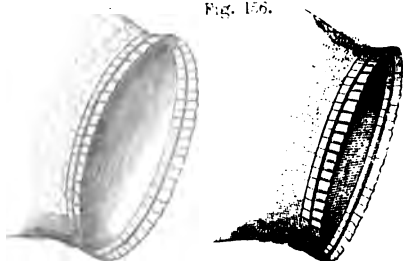
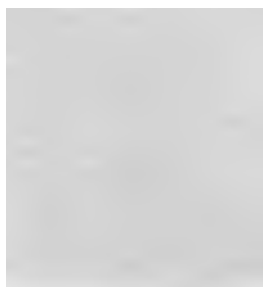


Fig. 150. Hair or spine of suchusa, showing how hard material is deposited. After Mr. Wenham.  
 Fig. 151. Diagram to show directions of currents in cells of Vallisneria. After Mr. Hunt.  
 Fig. 152. Portion of shell of pleurosigma. After Mr. Hunt.  
 Figs. 153, 154, and 155. Shells of rare diatoms. After Mr. Roper.  
 Fig. 156. Portions of the egg of the common bed bug. The speculum which covers the orifice removed. Stereoscopic drawing. After Mr. Wenham.

[To follow Pl. XXXIII.]



become converted into the tissue of which the spine is composed. Mr. Wenham well describes this process as follows: a dense current of corpuscles travels along one wall of the spine constantly returning by the opposite side *b, b*. At *c*, where the deposition occurs, there is a considerable accumulation, and at the boundary where they are converted into the substance of the spine a number are seen to be adherent. Often in specimens of this plant the deposition has been so rapid that there was not sufficient time for the complete condensation of the component corpuscles. In these instances a number of them have been caught and loosely enclosed in one or more cavities, as shown at *d, d*. The walls of these containing cavities do not possess a definite outline because these are lined with corpuscles in all their different stages of transition.

The course which the current takes in the cells of *vallisneria*, *anacharis*, &c., is not perfectly regular, and is indicated by the arrows in Fig. 157, after Dr. Branson.

If seaweed is to be preserved permanently, it should be allowed to soak for some time in pure water. Small pieces may then be removed and transferred to glycerine, in which fluid they may be preserved permanently after having been allowed to soak for some time. Some of the most beautiful vegetable preparations which I have seen have been mounted in glycerine. The mixture of gelatine and glycerine (§ 106) and the gum of glycerine (§ 107) will also be found good media for mounting many vegetable structures, and chloride of calcium forms a useful preservative fluid in many instances. Creosote fluid, carbolic acid water, very dilute spirit and water, and even simple distilled water will preserve some vegetable tissues for a great length of time. The pith of the stem of various plants, the epidermis, and many other vegetable tissues may be preserved as dry objects very satisfactorily.

**164. Vivaria.**—Vegetable structures and animals of various kinds may be kept for microscopical examination in small glass jars and under glass shades, which are now to be purchased at the glass shops in every part of London. Different forms are represented in Plate XXXIII, Fig. 146.



EXAMINATION AND PRESERVATION OF OBJECTS IN CANADA  
BALSAM.

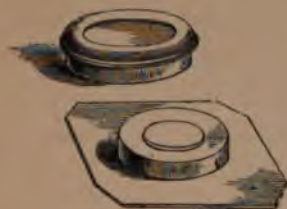
**165. Examination in Canada Balsam.** — This resinous substance has long been a favourite medium for the preservation of microscopical specimens, on account of its penetrating and highly refracting powers. Turpentine possesses very similar properties, but from being a limpid fluid, it is far less useful than Canada balsam. All preparations to be mounted in Canada balsam must be thoroughly dried first. The desiccation must be effected by a temperature of not more than from 100 to 200 degrees. For the purpose of drying tissues, we may employ the water-bath alluded to in § 73, or we may place the specimen under a bell-jar close to a basin of strong sulphuric acid or chloride of calcium, which substances have the power of absorbing moisture in an eminent degree. Many textures in process of drying include a number of air-bubbles in their interstices, and it is often very difficult to remove these. To effect this object, the preparation may be allowed to soak some time in turpentine, and the removal of the air is often much facilitated by the application of a gentle heat. If the air cannot be removed in this manner, the preparation immersed in turpentine, may be placed under the receiver of an air pump. As the pressure is removed the air rises to the surface and the fluid rushes in to supply its place. A convenient and simple form of air pump is represented in Plate XXXV, Fig. 160.

When the specimen has been thoroughly dried, and the air removed, it may be slightly moistened with turpentine before it is placed in the balsam.

Oil is an advantageous highly refracting medium for examining certain structures in. The entozoa which may often be obtained from the oily sebaceous matter squeezed from the follicles of the skin of the nose or scalp should be immersed in oil (Plate XXXII, Fig. 145). They can generally be found in the wax from the ear.

In mounting a thin section of bone in Canada balsam, the following steps are taken: the glass slide having been warmed upon the brass plate, a small quantity of Canada balsam is removed upon the end of a piece of iron wire. By gently warming it, it becomes perfectly fluid, and may be allowed to drop in its

Fig. 157



§§ 133, 154.

Fig. 159.



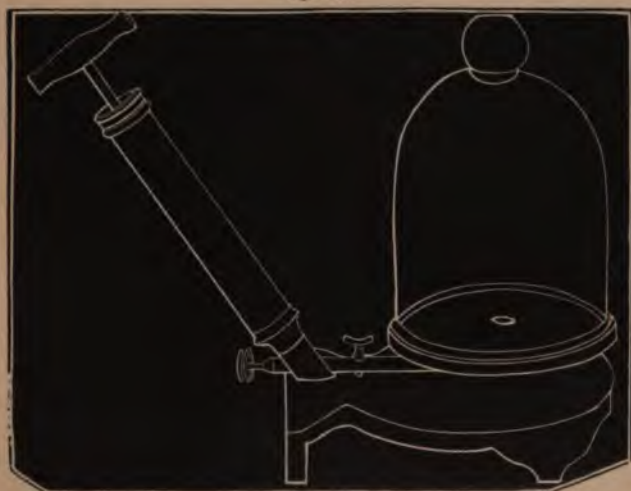
§ 161.

Fig. 158.



218.

Fig. 160.



§ 166.

Fig. 157. Animalcule cage for examining deposits from fluids. Fig. 158. Arrangement for heating an object while it is being examined. Fig. 159. Instrument for pressing down the thin glass cover while cement is drying. Rev. J. Isbell. Fig. 160. Small air-pump for removing air from the interstices of a tissue.

[To face page 98.]



proper place upon the glass slide. Or the metal pot containing the Canada balsam may itself be warmed, and a drop of the fluid balsam placed upon the slide. The preparation is now taken with a needle and placed in the drop of balsam, so that it may be thoroughly wetted by it in every part. Upon the surface of the balsam, a few air-bubbles may be observed, and by moving the slide from side to side, with a slight rotatory movement while the balsam is quite fluid, the bubbles may be seen to collect in one spot upon the surface. They may be made to burst by the application of a warm needle, or completely removed by touching them with a cold wire to which the balsam including them will adhere. All bubbles having been removed, the thin glass, which has been perfectly cleaned and slightly warmed on the brass plate, is taken in a pair of forceps,—and gently allowing one side of it to come in contact with the balsam,—is permitted to fall very slowly upon the specimen, in such a manner that the balsam gradually wets the thin glass, without including air-bubbles. It is then pressed down slightly with a needle, and the slide placed in a warm place. The superabundant balsam may be scraped away, and the preparation when cold, cleaned with a little turpentine, and a soft cloth, or piece of wash-leather.

The feet and hard parts of the fly and other insects, and the ova of small insects may be mounted in Canada balsam. The shells and hard parts of the covering of many of the lower animals, the palates of various mollusks, such as the limpet, and many fresh-water species, the coriaceous coverings of insects, their antennæ, stings, eyes, feet, wings, and scales of their wings, the tracheæ penetrating every part of their organism with their spiracles or external openings, and in some cases the entire insects themselves, the scales of fishes, sections of bone, teeth, horn, hoofs, claws, nails, specimens of various kinds of hair, are examples of objects derived from the animal kingdom which may be mounted in this manner.

**166. Examination of Hard Tissues.**—Bone may be made to present very different characters in Canada balsam, according to the manner in which it is mounted. Thus, in every part of one specimen, small black spots of irregular shape may be seen. From these a number of minute dark lines radiate, and inosculate pretty freely with corresponding lines from other spots.



In another no such appearance may be observable, and the entire section may appear clear, and its structure nearly uniform throughout. The first appearance is produced by mounting the section in old viscid balsam; the second by its immersion in fluid balsam, after having been previously wetted with turpentine.

The cause of these differences is interesting and worthy of attentive study. The little black spots (lacunæ) and dark lines (canaliculi) were originally considered to be small solid bodies, and the spots were improperly termed *bone corpuscles*. In truth, they consist of little cavities in the bony tissue, containing air. In the second specimen the highly refracting oil of turpentine has passed up the canaliculi and entered the lacunæ, thus rendering them invisible. These cavities, in the fresh bone, contain masses of germinal matter or "nuclei," but when the bone becomes dry, this moist material dries up, and air rushes into the lacunæ and canaliculi to supply its place. The great difference between the refracting power of the air contained in these little cavities, and the osseous tissue in which they are contained, gives rise to their dark appearance.

The general description given of the structure of bone applies only to the dead and dried tissue.

**167. Air Bubbles** in water have a very wide dark outline: indeed, small air bubbles take the form of round black spots. This appearance is very characteristic, and every observer ought to be thoroughly familiar with it. Air bubbles of various sizes are represented in Plate XXXVI, Fig. 161.

**168. Oil Globules** also present a peculiar and well-known appearance. The outline is sharp, and dark, and well-defined, but not nearly so wide as that of the air bubble, because the difference of the refractive power between the oil and the fluid, although very great, is much less than that which exists between the air and the fluid medium which contains it. Every one should compare carefully air bubbles with oil globules under the microscope. Oil globules within cells and free oil globules are seen in Plate XXXVI, Figs. 162, 166, oil globules of various sizes, as seen in milk, in Figs. 163, 164. Every observer should be familiar with the microscopical appearance of oil globules of different kinds. Certain kinds of fatty matter contain much crystalline fat, as stearine or margarin, which crystallizes



Fig. 161.



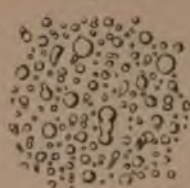
§ 167.

Fig. 162.



§ 168.

Fig. 163.



§ 164.

Fig. 165.



§ 169.

Fig. 166.



§ 168.

Fig. 167.



§ 168.

Fig. 167.



§ 170.

Fig. 168.



§ 168.

Fig. 170.

Fig. 169.



§ 168.



§ 168.

Fig. 161. Air bubbles of various sizes in water. Fig. 162. Oil globules, some free, others in envelopes. Figs. 163 and 164. Oil globules from milk. In some the investment of casein is dissolved and they are coalescing. Fig. 165. Blood corpuscles. Fig. 166. Liver cells containing oil-globules. In the centre is seen a collection of oil-globules not surrounded by any envelope. Fig. 167. The yeast fungus in various stages of development, after Dr. Hassall. Fig. 168. Crystals of stearic acid. Fig. 169. Crystals of margaric acid separating from the oily fat or oleins. Fig. 170. Crystals of margaric acid.

[To face page 169.]



spontaneously from the more oily fatty matters. And by the action of acids and other agents, many fats are decomposed, and the crystalline fatty acids are set free. Crystals of fats and fatty acids, are represented in Plate XXXVI, Figs. 168, 169, 170.

**169. Blood Corpuscles** or globules from the human subject, are represented in Plate XXXVI, Fig. 165. Their general characters, and especially their colour and refractive power, should be contrasted with oil globules of different kinds, air bubbles, and fungi—particles of common mildew or yeast (Fig. 167) for instance. The student should carefully examine specimens of these bodies. Blood corpuscles are readily obtained by pricking the finger. A very thin stratum of the fluid is alone required. The blood corpuscles of various animals should also be carefully examined, especially those of the frog, of some fish, and bird.

**170. Fungi.**—The sporules of some fungi very closely resemble blood corpuscles, and have been mistaken for blood corpuscles. The common yeast fungus, in different stages of growth, is represented in Plate XXXVI, Fig. 167. See also my "*Archives*," Vol. II, p. 49.

#### ON THE SEPARATION OF DEPOSITS FROM FLUIDS.

In order to ascertain the nature of a deposit suspended in a fluid, there are two or three important but very simple processes to be borne in mind. The first object is to separate the deposit as much as possible from the surrounding fluid, to collect it into a small space. Diffused as it often is through a large bulk of fluid, the observer would scarcely be surprised if he failed to find what he was looking for when a drop of the fluid was placed under the microscope.

**171. Conical Glasses.**—In order to collect the deposit for microscopical examination, the fluid is placed in a conical glass, the lower portion of which is narrow, but which at the same time does not terminate in a point but in a slightly-rounded extremity. After standing for some hours, the deposit falls to the narrow portion of the glass, and may be removed with the pipette. A useful form of conical glass is represented in Plate XXXVII, Fig. 171.

172. The Pipette consists of a glass tube, about ten inches in length, the upper extremity being slightly enlarged, so that the finger may be conveniently applied to it, and the lower orifice contracted, so as to be about one-tenth of an inch in diameter. It is convenient to have a ridge around the glass tube, about three inches from its upper extremity (Plate XXXVII, Fig. 172).

173. Removing the Deposit with the Pipette.—The removal of the deposit is exceedingly simple. The pipette is held by the middle finger and thumb, while the index finger is firmly applied to its upper extremity. The point is next plunged beneath the surface of the fluid and carried down to the deposit, a portion of which will rush up the tube if the pressure of the finger upon the upper extremity be slightly diminished. The deposit having entered the tube, the pressure is re-applied, and the deposit contained in the pipette can be removed from the fluid (Fig. 171).

174. Separation of Deposit when very Small in Quantity.—Where the deposit is exceedingly small in quantity, and diffused through a great bulk of fluid, a slight modification of the above plan must be resorted to. The pipette containing as much of the deposit as can be obtained, is removed from the glass vessel containing the fluid. Its contents are prevented from escaping by the application of the finger to its lower orifice. The upper extremity is then occluded with a small cork. Upon now removing the finger from the lower orifice, of course no fluid will escape. The pipette is allowed to stand with its mouth downwards upon the glass slide, in which position it may be permitted to remain some hours, either being suspended with a string or allowed to lean against some upright object. It is obvious that under these circumstances the most minute deposit contained in the fluid will gravitate to its lower part, and be received upon the slide, without the escape of much of the fluid. Or the sediment, having been allowed to subside in a conical glass, may be poured into a very narrow test tube. Upon a glass slide being applied to the open end, the tube may be inverted, and the deposit will gradually be deposited upon the slide. The arrangement will be understood by reference to Fig. 175.

According to either of the above methods any insoluble substances diffused through fluids can be easily collected for the



purposes of examination. In collecting shells of the diatomaceæ for microscopical examination they are often diffused through a considerable quantity of water, allowed to subside, and obtained in the manner above described.

Siliceous shells of certain diatoms are represented in Plate XXXIV, Figs. 152, 153, 154, 155. There is much difference of opinion as to the cause of the markings in many of these. Mr. Hunt considers the dots on pleurosigma represented in Fig. 152, as elevations not depressions (*"Mic. Journ."* Vol. III, p. 175).

**175. Examination of Infusoria, &c.**—Suppose the student desires to submit some of the animalcules in water to microscopical examination, he would proceed as follows. A drop of the water must be removed with a pipette, or upon a glass rod, or with the finger, and placed upon the glass slide. A bristle or thin piece of paper is placed in such a position as to prevent the thin glass from coming into too close contact with the slide; or the drop may be placed in a Brunswick black, or thin glass cell; or the animalcule cage previously described (Plate XXXV, Fig. 157, Plate XI, Fig. 32) may be used with advantage. By the latter instrument the larger infusoria may be kept still in a particular position for the purposes of examination.

**176. Vorticellæ and Rotifers**, or wheel-animalcules, may often be obtained by placing a small piece of a plant which has been allowed to remain in the same water for some time, with a drop of the fluid, in a glass cell, observing the precautions before alluded to (§ 159). These organisms are often found attached to the edges of the plant in considerable number.

**177. Zoophytes.**—Fresh-water and marine Zoophytes, too large to be placed in the small cells, may be examined in flat watch glasses, or in one of the larger cells alluded to in § 128.

These may be examined with low powers (two inch, one inch) without any thin glass cover, but where the higher powers are employed a piece of thin glass must be applied in such a manner as to cover that part of the vessel in which the animals are situated, without preventing a certain proportion of the fluid from being exposed to the air; for if exposure to the air were prevented, the animals would soon exhaust all that dissolved in



the small quantity of water in which they were imprisoned, and die of suffocation.

**178. On Separating the Coarse from the Finer Particles of a Deposit.**—Many deposits, by being diffused through a large quantity of water, may be divided into several portions. The fluid, with substances suspended in it, is well stirred, and, after being allowed to stand for a very short time, all but the deposit is poured off into another vessel. In this the fluid is again allowed to stand for a short time, and again poured off. This process may be repeated several times. In the first glass, only the coarser particles will be found; in the second, slightly finer particles; in the third, still finer ones, and so on; a longer period being allowed for the subsidence in each successive case.

The coarse particles may also often be separated from finer ones by straining the deposit through muslin. Various preservative solutions, which I have already described, are applicable for preserving deposits from fluids. Many, again, may be mounted in Canada balsam.

**179. Method of obtaining the Siliceous Skeletons of Lower Organisms.**—The siliceous remains of the diatomaceæ may be separated from guano and other deposits as follows. The organic matter and carbonate and phosphate may be removed by boiling in nitric acid, and the remaining deposit diffused through water and collected as before described, but I much prefer to destroy the organic matter by burning the deposit in a platinum basin, and allowing it to remain for some hours at a red heat until the black carbonaceous matter has burnt off, leaving a pure white ash. The phosphates and carbonates may be removed with dilute nitric acid, and the deposit washed. In this way the shells are not so liable to be broken as they are when the deposit is boiled for some time in strong acid.

**180. Wash-bottle.**—In many operations the wash-bottle used by chemists is of great use, as by it a stream of water of any required degree of force can be easily directed to any particular point, either for the purpose of washing away foreign particles, or for removing part of the deposit itself. The wash-bottle is also of great use in preparing sections of soft tissues for observation. It is made by inserting a cork into an ordinary half-pint

PLATE XXXVII.

Fig. 171.



§§ 171, 173.

Fig. 172.



§ 173.

Fig. 173.



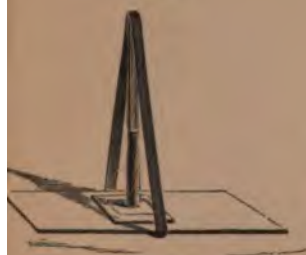
§ 180.

Fig. 174.



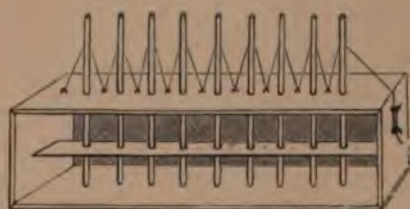
§ 159.

Fig. 175.



§ 174.

Fig. 176.



§ 161.

Fig. 171. Illustrates the mode of using the pipette.

Fig. 172. Pipettes made of glass tubing.

Fig. 173. Wash bottle.

Fig. 174. To illustrate the manner in which the thin glass is allowed to fall gradually upon an object mounted in fluid.

Fig. 175. Shows the manner in which a very small quantity of deposit may be obtained from a fluid, by placing it in a test tube, and inverting it over the glass slide. It is kept in position by an India-rubber band shown in the drawing.

Fig. 176. Arrangement for exerting continued pressure upon the glass cover while cement is drying.

(To face page 104.)



bottle. Through the cork pass two tubes, bent at the proper angle. The longest terminates in a capillary orifice, while its other extremity reaches down to the bottom of the bottle. The shorter tube reaches only to the lower part of the cork (Plate XXXVII, Fig. 173). By blowing through the shorter tube, air is made to press upon the surface of the water, which is thus driven up the longer tube and out at its capillary orifice.

The observer will also require a stock of *small tubes*, about two inches in length and a quarter of an inch in diameter, and several small *watch glasses*, of different sizes.

**181. Of Keeping Preparations in the Cabinet.**—Preparations mounted in the dry way, or in Canada balsam, may be kept upright, arranged in grooves, but all preparations mounted in fluid must be allowed to lie perfectly flat, otherwise there will be great danger of leakage. Cabinets holding several hundred specimens arranged in this manner may now be purchased of the microscope makers for a very small sum, but if the observer is only provided with deep drawers, they may be made available for the purpose, by having a number of shallow trays made to fit them accurately. Each preparation should be named as soon as it is put up, and it is convenient to keep a number of small gummed labels always at hand for this purpose. Once or twice in the year a new layer of Brunswick black should be applied, and the specimens carefully examined to see that no leakage has occurred.





## CHAPTER VII.

OF INJECTING.—*Natural and Artificial Injections—Transparent and Opaque Injections—Instruments required for making Injections—Syringe, Pipes, Stop-cocks, Bull's-nose Forceps, Needle for passing the Thread round the Vessel.*  
 OF OPAQUE INJECTIONS.—*Injecting Cans—Size—Colouring Matters—Vermilion—Chromate of Lead—White Lead—Size of the Particles of the Colouring Matter used.*  
 OF TRANSPARENT INJECTIONS.—*Injecting with Plain Size—Colouring Matters—Gerlach's Carmine Injecting Fluid.—Advantages of employing Prussian Blue—Composition of the Prussian Blue Fluid for making Transparent Injections—On injecting different Systems of Vessels with Opaque and Transparent Injections—Acid Carmine Fluid—Mercurial Injections.* INJECTING THE LOWER ANIMALS.—*Mollusca—Insects.* OF THE PRACTICAL OPERATION OF INJECTION.—*Of Injecting the Ducts and Secreting Follicles of Glands—Of preparing Portions of Injected Preparations for Microscopical Examination.*

THE arrangement of the minute vessels or capillaries distributed to various textures is not to be demonstrated in all instances by the usual methods of investigation, in consequence of the transparency of the walls of the tubes. Indeed, in an ordinary examination of a tissue in the microscope, one often fails to detect the least trace of any structure which would be regarded as consisting of distinct tubes or vessels. Some authorities even yet maintain the opinion, that the capillaries are to be looked upon in the light of mere channels in the interstices of the tissues, rather than as tubes, with their own proper walls. If this opinion were correct we should hardly expect to meet with the perfectly circular outline which the section of an injected capillary vessel frequently presents; nor should we be able to obtain capillaries isolated from other tissues.

**182. Natural and Artificial Injections.**—Sometimes the capillary vessels remain turgid with blood after the death of the animal, and a *natural injection* results. Natural injections, however, are accidental and cannot be obtained in the case of every texture. In order, therefore, to investigate the arrangement of the vessels, it is necessary to resort to the process of *artificial injection*, which consists simply of forcing into a vessel of convenient size a certain quantity of coloured material, which, after passing along the large trunk, shall penetrate into the smallest vessels and even return by the veins. The colouring matter employed may be *opaque* or *transparent*. In the first case the injected preparation can only be examined by *reflected light* as an *opaque object* (§ 23), while transparent injections may be subjected to examination either by the aid of *transmitted light* (§ 30), or by *reflected light*. Examples of opaque and transparent injections in which different substances have been employed as colouring matters, can be purchased at all the microscope makers, but every student is recommended to prepare injected preparations himself.\*

**183. Instruments required for Making Injections.**—The different instruments required for making artificial injections are the following:—An *injecting syringe*, of about the capacity of one ounce or even half an ounce (Plate XXXVIII, Fig. 184). The piston of the injecting syringe should be covered with two pieces of leather, which may be very easily removed and replaced (Fig. 179). The first piece, *a*, is applied and screwed down with a brass button, *b*. The piston is then passed down the tube and forced out at the lower opening. The second piece of leather, *c*, is then put on, and fixed in its place with another button, *d*. In the syringes now made for me by Mr. Matthews, the piston consists entirely of metal. I have found syringes of this description work exceedingly well, and the necessity for re-leathering is obviated, but they are rather expensive.

*Pipes*, of different sizes, to insert into the vessels (Fig. 182). The tubes of the smaller pipes should be made of silver.

*Corks*, of the form represented in Fig. 177, for the purpose of

\* Most beautiful injections are made by Mr. Hett, Mr. Rainey, Mr. Norman, Mr. Webb, of Birmingham; and injections of every kind may be purchased of Mr. Matthews, Messrs. Smith and Beck, and others.

Fig. 177.



Fig. 183.

§ 183.

Fig. 178.



§ 183.

Fig. 179.



§ 183.

Fig. 180.



§ 184.

Fig. 181.



§ 183.



§ 204.

Fig. 182.



§ 183.

Fig. 183.



§ 183.

Fig. 177. Corks for stopping the pipe when the syringe is being refilled. Fig. 178. Bull's-nose forceps for closing an open vessel to prevent the escape of the injection. Fig. 179. Shows the manner in which the piston of the syringe is made. *a, c*, pieces of leather. Fig. 180. Injecting can for heating size. It may also be used as a water bath for drying objects, or for conducting evaporation. Fig. 181. Performing the operation of injecting. Fig. 182. Stopcock and injecting pipe, which fit on to the syringe. Fig. 183. Needle for passing thread round a vessel, the cut end of which is to be tied on an injecting pipe. Fig. 184. Syringe for injecting small animals, &c.

[To face page 108.]





plugging the pipes while the syringe is being filled with injecting fluid. A stopcock (Fig. 182), is also useful for the same purpose.

*Forceps*, of the form shown in Fig. 178, which are known to surgical instrument makers as *bull's nose forceps*, for stopping up any vessels through which the injection may escape accidentally.

*A Needle*, of the form of the *aneurism needle* used by surgeons, for passing the thread round the vessel to tie it to the pipe which is inserted into it (Fig. 183). This needle may be made of an ordinary darning needle which has been carefully bent round after having been heated in the flame of a lamp. The *thread* which is used should be strong but not too thin, as there would be danger of its cutting through the coats of the vessel.

#### OF OPAQUE INJECTIONS.

Although, as a means of making out new points in the structure of living beings, I feel sure that the old system of injection is useless—as some may desire to prepare specimens, I leave the directions for making these injections in this edition. The observer must, however, not suppose that he will be able to add to our knowledge, however great an adept he may be at this difficult process. To make a perfect vermilion injection, requires skill that any one may be proud to possess, but all that was to be made out by the process has probably been discovered already.

**184. Injecting Cans.** — Size or gelatine is used as the material in which the coloring matter is suspended. It must be melted in a water-bath and strained immediately before use. The copper injecting can forms a very convenient arrangement for melting the gelatine. There are five cans in the bath, seen in Fig. 180, Plate XXXVIII, so that injection may be very conveniently transferred from one into the other, while all may be kept warm over an ordinary lamp.

**185. The Size** should be of such a strength as to form a tolerably firm jelly on cooling. If gelatine is employed it must be soaked for some hours in cold water before it is warmed. About an ounce of gelatine to a pint of water will be sufficiently strong, but in very hot weather it is necessary to add a little more gelatine. It must be soaked in part of the cold water until



it swells up and becomes soft, when the rest of the water, made hot, is to be added. Good gelatine for injecting purposes may be obtained for two shillings a pound.

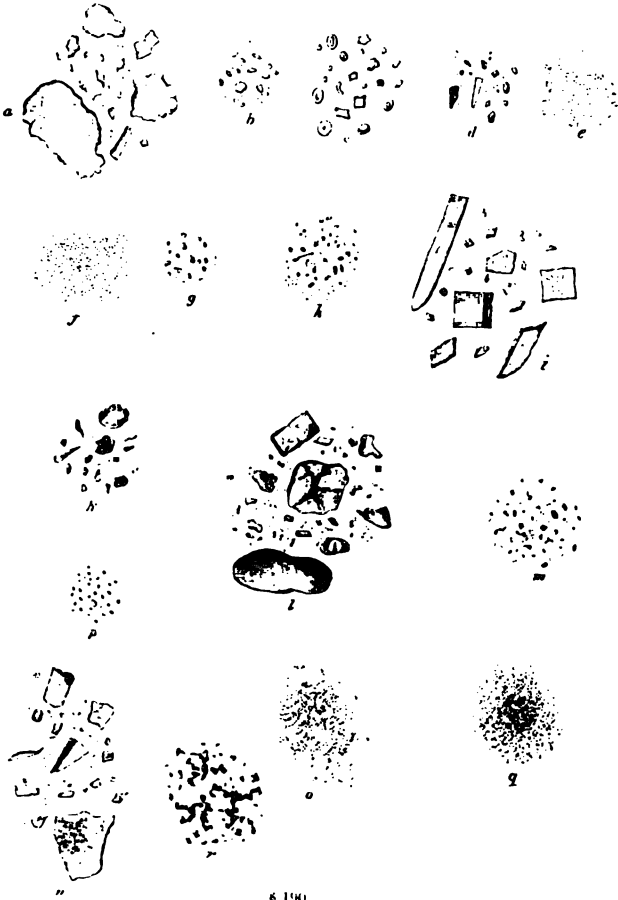
**186. Coloring Matters.**—The most usual coloring matters employed for making opaque injections are the following—*Vermilion*, *Chromate of Lead*, and *White Lead*. Of these, vermilion affords the most beautiful preparations, but chromate of lead properly prepared is much cheaper, and it may be obtained in a state of more minute division. White lead forms a good coloring matter, but its density, and its tendency to become brown when exposed to the action of sulphuretted hydrogen, formed in the decomposition of the tissues, are objections to its use.

**187. Vermilion** of sufficiently good quality can be purchased of all artists' colormen for six or eight shillings a pound. If upon microscopical examination a number of very coarse particles be found in the vermilion, it will be necessary to separate these by washing in water in the manner described in § 178.

**188. The Chromate of Lead** is prepared by mixing cold saturated solutions of acetate of lead and bichromate of potash. The yellow precipitate is allowed to settle, and after pouring off the clear solution of acetate of potash resulting from the decomposition, it is shaken up with water, again allowed to settle and mixed with strong size or gelatine. After being strained through muslin the mixture may be injected into the vessels.

**189. The Carbonate of Lead or White Lead** is prepared by mixing saturated solutions of acetate of lead and carbonate of soda. The precipitate is to be treated as the former one and mixed with size.

There should always be plenty of coloring matter in the size, otherwise the vessels do not appear to be uniformly filled, and it is better to employ a small syringe rather than a large one, as there is not so much chance of the coloring matter separating from the size before the mixture is forced into the vessels. In all cases the colouring matter is to be well mixed with the size, and the mixture strained through muslin immediately before use.



§ 190

Thousands of an Inch.  
Scale 1 2 3 4 5 6

Colouring matter used for injecting, showing the comparative size of the particles. *a*. Precipitated chalk purchased in a dry state. *b*. Chalk recently precipitated. *c*. Whitening. *d*. Prussian blue, as purchased. *e*. Recently precipitated Prussian blue. *f*. Freshly precipitated carbonate of lead. *g*. Dried carbonate of lead. *h*. Freshly precipitated biniodide of mercury. *i*. Dried biniodide of mercury. *k*. Indigo. *l*. Vermilion, as purchased. *m*. Levigated vermilion. *n*. Pure carmine. *u*. Dried chromate of lead. *r*. Freshly precipitated chromate of lead (hot solutions of bichromate of potash and acetate of lead). *o*. Freshly precipitated chromate of lead (cold solutions). *q*. Lamp black.  $\times 215$ .

[To face page 110.]



**190. Size of the particles of the Coloring matter used.**—The size of the ultimate particles of the different substances employed in making opaque injections is represented in Plate XXXIX, and if the different figures be compared with one another, it will be observed that those coloring matters which have been recently prepared are in a much more minute state of division than those which have been kept for some time. The appearances represented were obtained by examination with a power of 215 diameters.

Opaque injections are represented in Plate XL, Figs. 186, 187, the latter being an injection of capillary vessels,

#### OP TRANSPARENT INJECTIONS.

**191. Advantages of Transparent Injections.**—For many years I have abandoned the old plan of making injected preparations in favour of such transparent injecting fluids as are miscible with water in all proportions, which besides the coloring matter contain solutions which exert a preservative action upon the tissue with which they come in contact, or a special influence in rendering certain elements of the tissue more transparent or opaque. By this new plan of injection most important advantages are gained.

1. The vessels are injected with coloring matter.
2. The tissues are preserved from decomposition or change by the action of the fluids of the body which are washed out by the injection.
3. Certain tissues may be rendered more distinct.
4. Every structural element of the tissue can be seen as well as the vessels.
5. Tissues prepared by this process can be subjected to examination, by powers magnifying more than 5,000 diameters.
6. All tissues may be mounted in aqueous preservative solutions, and the most delicate structures are retained in their integrity.
7. By this process of injection alone can the alteration of the most delicate tissues, which occurs very soon after death, be prevented.

It will be seen that several important principles are involved in this new process, which will be more fully enunciated in

**Chapter X.** Of late years carmine fluid has been much used for transparent injections, especially in Germany; but although many of these specimens are very beautiful, I am not aware that many new facts have been revealed by the process. As the specimens are mounted in balsam, the structure of the tissues external to the vessels is completely lost. I would again strongly urge that all that is to be learnt by such modes of preparation, has already been learnt, and that for more minute investigation it is absolutely necessary that the tissue be prevented from undergoing post mortem change, and that it be preserved in *viscidi aqueous fluids*. Drying the tissue destroys the structure, and the only mode of preparation by which injected textures can be subjected to examination by the highest powers, and by which all the several structures entering into their composition can be displayed in the same specimen, is that which I am advocating in conjunction with the staining process described in Chapter X.

In order to inject the vessels for examination by transmitted light several different substances may, however, be used as injecting fluids: but if it is desired to study the tissues as well as the arrangement of the vessels, the points just adverted to must be borne in mind.

**193. Injection with Plain Size.**—A tissue which has been injected with plain size, when cold is of a good consistence for obtaining thin sections, and many important points may be learned from a specimen prepared in this manner which would not be detected by other modes of preparation. A mixture of equal parts of gelatine and glycerine is, however, much to be preferred for this purpose, and the specimen thus prepared is sure to keep well. Very thin sections of spongy tissues like the lung may be made after injection with strong gelatine.

**194. Coloring Matters for Transparent Injections.**—The chief coloring matters used for making transparent injections are *carmine* and *Prussian blue*. The former may be prepared by adding a little solution of ammonia (liquor ammoniæ) to the carmine, and diluting the mixture until the proper colour is obtained, or it may be diluted with size. The latter by adding a persalt of iron to a solution of ferrocyanide of potassium.

**195. Gerlach's Carmine Injecting Fluid.**—Prof. Gerlach was



the first who used a carmine injecting fluid. The beautiful carmine injections now made in Germany are prepared with this fluid, or a slight modification of it. I take the receipt from the excellent work of Dr. Frey ("*Das Mikroskop*," 1863).

Carmine . . . . .	77 grains.
Water . . . . .	70 grains.
Liquor Ammoniaë . . . . .	8 drops.

The carmine is to be dissolved in the ammonia and water, and the solution left for some days exposed to the air, and then mixed with pure gelatine, made by dissolving a drachm and a-half of good gelatine in a drachm and three quarters of water. Lastly, a few drops of acetic acid are added to the mixture, which is injected warm.

Dr. Carter recommends the following carmine injection (my "*Archives*," Vol III, page 288):—

Pure Carmine . . . . .	1 drachm.
Liq. Ammon. fort. . . . .	2 drachms.
Glacial Acetic Acid . . . . .	1 drachm 26 minims.
Solution of Gelatine (1 to 6 of water) . . . . .	2 ounces.
Water . . . . .	1½ ounces.

Dissolve the carmine in the solution of ammonia and water, and filter if necessary. To this add an ounce and a half of the hot solution of gelatine, and mix thoroughly. With the remaining half ounce of gelatine solution mix the acetic acid, and then drop this little by little into the carmine solution, stirring briskly during the whole time.

**196. Advantages of Employing Prussian Blue.** — *The Prussian blue fluid* consists of an insoluble precipitate, so minutely divided, that it appears like a solution to the eye. The particles of freshly prepared Prussian blue are very much smaller than those of any of the coloring matters employed for making opaque injections. For many years I have employed Prussian blue as the injecting fluid, and according to my experience it possesses advantages over every other coloring matter. It is inexpensive, — may be injected cold, — the preparation does not require to be warmed, — no size is required, — it penetrates the capillaries without the necessity of applying much force, — it does not run out when a section is made for examination, — neither do any

particles which may escape from the larger vessels divided in making the section, adhere to it and thus render the section obscure,—a structure may be well injected with it in the course of a few minutes. Specimens prepared in this manner may be preserved in any of the ordinary preservative solutions, or may be dried and mounted in Canada balsam (but I give the preference to glycerine, § 100, or glycerine jelly, § 106), and they may be examined with the highest magnifying powers. After having tried very many methods of making this preparation I have found the following one to succeed well. For very fine injections the mixture may be diluted by adding three ounces of glycerine.

*Composition of the Prussian Blue Fluid for Making Transparent Injections:—*

Glycerine . . . . .	2 ounces.
Wood naphtha, or pyroacetic spirit . . . . .	1½ drachms.
Spirits of wine . . . . .	1 ounce.
Ferrocyanide of potassium . . . . .	12 grains.
Tincture of sesquichloride of iron . . . . .	1 drachm.
Water . . . . .	3 ounces.

The ferrocyanide of potassium is to be dissolved in one ounce of the glycerine, and the tincture of sesquichloride of iron added to another ounce. These solutions should be mixed together very gradually, and well shaken in a bottle. *The iron being added to the solution of the ferrocyanide of potassium.* When thoroughly mixed, these solutions should produce a dark blue mixture, in which no precipitate or flocculi are observable. Next, the naphtha is to be mixed with the spirit, and the water added very gradually, the mixture being constantly shaken in a large stoppered bottle. The tincture of sesquichloride of iron is recommended because it can always be obtained of nearly uniform strength. It is generally called the *muriated tincture of iron*, and may always be purchased of druggists. In cases in which a very fine injection is to be made for examination with the highest powers, half the quantity of iron and ferrocyanide of potassium may be used.

Mr. Richardson recommends Turnbull's blue instead of Prussian blue. Protosulphate of iron and ferrocyanide of potassium are the salts to be used. The mixture is made with glycerine upon the same principles as my own fluid. Mr. Richardson states that the colour of the Turnbull's blue is brighter, and is retained

longer than is the case with the Prussian blue. The latter does not, however, lose its colour if a little free acid is present in the fluid in which the specimen is preserved. I have many specimens injected with Prussian blue, which have retained their colour perfectly for more than ten years. The advantage of the Prussian blue over other fluids is, that the ingredients required to make it are very cheap, and can be readily obtained everywhere. Capillaries injected with Prussian blue fluid under high magnifying powers are represented in Plates XXVIII, XL.

I would most earnestly recommend all who are fond of injecting to employ transparent injections, and to endeavour by trying various transparent coloring matters, to discover several which may be employed for this purpose, for I feel sure that by the use of carefully prepared transparent injections, many new points in the anatomy of tissues will be made out.

**197. Of Injecting Different Systems of Vessels with different Opaque Injections** — It is often desirable to inject different systems of vessels distributed to an organ with different colours, in order to ascertain the arrangement of each set of vessels and their relation to each other. A portion of the gall-bladder in which the veins have been injected with white lead, and the arteries with vermilion, forms a beautiful preparation. Each artery, even to its smallest branches, is seen to be accompanied by two small veins, one lying on either side of it. A beautiful injection of the gall-bladder is represented in Plate XL, Fig. 186.

In an injection of the liver, four sets of tubes may be injected as follows:—The artery with vermilion, the portal vein with white lead, the duct with Prussian blue, and the hepatic vein with lake. There are many opaque coloring matters which may be employed for double injections.

**198. Of Injecting Different Systems of Vessels with Transparent Injections.**—The transparent injecting fluids which may be used for double injections, must have the same reaction. Thus the Prussian blue fluid, and the carmine solution without gelatine, given below, may be used for this purpose; but I have not yet been able to obtain other colours which answer so well as these. Good transparent yellow and green *acid* injecting fluids which might be used for double injections in cases in which the Prussian blue fluid was employed, are much to be desired.



**199. Acid Carmine Fluid.**—After trying a great many different combinations, I arrived at the following, which answers the purpose exceedingly well :—

Carmine . . . . .	5 grains.
Glycerine, with about eight or ten drops of acetic or hydrochloric acid . . . . .	} ½ ounce.
Glycerine . . . . .	
Alcohol . . . . .	1 "
Water . . . . .	2 drachms.
Ammonia, a few drops.	6 "

Mix the carmine with a few drops of water, and when well incorporated, add about five drops of *liquor ammonia*. To this dark red solution, about half an ounce of the glycerine is to be added, and the whole well shaken in a bottle. Next, very gradually, pour in the acid glycerine, frequently shaking the bottle during admixture. Test the mixture with blue litmus paper, and if not of a very decidedly acid reaction, a few drops more acid may be added to the remainder of the glycerine, and mixed as before. Lastly, mix the alcohol and water very gradually, shaking the bottle thoroughly after adding each successive portion, till the whole is mixed. This fluid, like the Prussian blue, may be kept ready prepared, and injections may be made with it very rapidly.

**200. Mercurial Injections** are not much used for microscopical purposes although mercury was much employed formerly for injecting lymphatic vessels and the ducts of glandular organs. The pressure of the column of mercury supersedes the necessity of any other kind of force for driving it into the vessels. The mercurial injecting apparatus consists of a glass tube, about half an inch in diameter and twelve inches in length, to one end of which has been fitted a steel screw to which a steel injecting pipe may be attached. The pipes and stopcocks must be made of steel, for otherwise they would be destroyed by the action of the mercury.

**201. Injecting the Lower Animals.**—The vessels of fishes are exceedingly tender, and require great caution in filling them. It is often difficult or quite impossible to tie the pipe in the

vessel of a fish, and it will be generally found a much easier process to cut off the tail of the fish, and put the pipe into the divided vessel which lies immediately beneath the spinal column. In this simple manner beautiful injections of fish may be made. In many cases, in which the vessels are too delicate to be tied, a good injection may be made by simply placing the pipe in the vessel. As the fluid is so cheap, a considerable loss is of no importance.

**202. Mollusca.**—(Slug, snail, oyster, &c.). The tenuity of the vessels of the mollusca often renders it impossible to tie the pipe in the usual manner. The capillaries are, however, usually very large, so that the injection runs very readily. In different parts of the bodies of these animals are numerous lacunæ or spaces, which communicate directly with the vessels. Now, if an opening be made through the integument of the muscular foot of the animal (as the snail), a pipe may be inserted, and thus the vessels may be injected from these lacunæ with comparative facility.

**203. Insects.**—Injections of insects may be made by forcing the injection into the general abdominal cavity, whence it passes into the dorsal vessel and is afterwards distributed to the system. The superfluous injection is then washed away, and such parts of the body as may be required, removed for examination.

#### THE PROCESS OF INJECTING.

**204. Of the Practical Operation of Injecting.**—It is generally stated that a successful injection cannot be made until the muscular rigidity which comes on shortly after death, and which affects the muscular fibres of the arteries as well as those of the muscles themselves, has passed off; but I have found that most perfect injections may be made before the muscles begin to contract, that is, within a few minutes after the death of the animal. All my fine injections have been made less than five minutes after death.

The steps of the process of transparent injection are very similar to those taken in making the opaque injections, except that when size is employed, the specimen must be placed in warm water until warm through, otherwise the size will solidify



in the smaller vessels and the further flow of the injecting fluid will be prevented. Soaking for many hours is sometimes necessary for warming a large preparation thoroughly, and it is desirable to change the water frequently. The size must also be kept warm, strained immediately before use, and well stirred up each time the syringe is filled.

In the first place the following instruments must be conveniently arranged :—

The syringe thoroughly clean and in working order, with pipes, stopcock and corks.

One or two scalpels.

Two or three pair of sharp scissors.

Dissecting forceps.

Bell's nose forceps.

Curved needle threaded with silk or thread, the thickness of the latter depending upon the size of the vessel to be tied.

Wash-bottle.

Injecting fluid in a small vessel.

Suppose the student is about to inject a frog. An incision is made through the skin, and the sternum divided in the middle line with a pair of strong scissors; the two sides may easily be separated, and the heart is exposed. Next the sac in which the heart is contained (pericardium) is opened with scissors and the fleshy part of the heart seized with the forceps; a small opening is made near its lower part, and a considerable quantity of blood escapes from the wound—this is washed away carefully by the wash-bottle (§ 180). Into the opening—the tip of the heart being still held firmly by the forceps,—a pipe is inserted and directed upwards towards the base of the heart to the point where the artery is seen to be connected with the muscular substance. *Before the pipe is inserted, however, a little of the injecting fluid is drawn up so as to fill it, for if this were not done, the air contained in the pipe would necessarily be forced into the vessels, and the injection would fail.*

The point of the pipe can with very little trouble be made to enter the artery. The needle with the thread is next carried round the vessel and the thread seized with forceps, the needle unthreaded and withdrawn, or one end of the thread may be held firmly, while the needle is withdrawn over it in the opposite direction. The thread is now tied over the vessel, so as to include the tip of the pipe only, for if the pipe be tied too far up

there is great danger of its point passing through the delicate coats of the vessel.

The nozzle of the syringe, which has been well washed in warm water before commencing, is now plunged beneath the surface of the fluid, the piston moved up and down two or three times, so as to force out the air completely, and the syringe filled with fluid. It is then connected with the pipe, which is firmly held by the finger and thumb of the left hand, with a screwing movement, a little of the injection being first forced into the wide part of the pipe so as to prevent the possibility of any air being included.

The pipe and syringe being still held with the left hand, the piston is slowly and gently forced down with a slightly screwing movement with the right, care being taken not to distend the vessel so as to endanger rupture of its coats. The handle of the syringe is to be kept uppermost, and the syringe should never be completely emptied, in case of a little air remaining, which would thus be forced into the vessel (Fig. 181, Plate XXXVIII). The injection is now observed running into the smaller vessels in different parts of the organism.

The student is recommended to practise the process by injecting the organs and animals in the order in which they are enumerated, and not to attempt the second until he has succeeded with the first. In all cases the operation is to be conducted patiently, and very slight pressure on the piston is to be exerted.

1. Kidneys of sheep or pig.—*Artery.*

2. Eye of ox.—*Artery.*—Two or three minutes will be time enough to make a complete injection. If the globe becomes very much distended by the injecting process, an opening must be made in the cornea which will leave room for the injection and permit the complete distension of the vessels.

3. Rat, mouse, frog.—*Injected from the aorta.*

4. Portion of intestine.—*Branch of artery.* All divided vessels being tied before commencing to inject. Plate XL, Fig. 189.

5. Liver. In one part, a *branch of duct*; in a second, a *branch of artery*; in a third, *portal vein*; and in a fourth, *hepatic vein*. The portal and hepatic vein, the artery and portal or hepatic vein, or the duct and portal vein may be injected with injections of different colours in one part.

205. Of Injecting the Ducts of Glands. — The modes of injecting which have just been considered, although applicable to

the injection of vessels, are not adapted for injecting the ducts and glandular structure of glands; for as these ducts usually contain a certain quantity of the secretion, and are always lined with epithelium, it follows that when we attempt to force fluid into the duct, the epithelium and secretion must be driven towards the secreting structure of the gland, which is thus effectually plugged up with a colorless material, and there is no possibility of making out the arrangement of the parts. In such a case it is obviously useless to introduce an injecting fluid, for the greatest force which could be employed would be insufficient to drive the contents through the basement membrane, and the only possible result of the attempt would be rupture of the thin walls of the secreting structure and extravasation of the contents. As I have before mentioned, partial success has been obtained by employing mercury, but the preparations thus made are not adapted for microscopical observation.

I had long felt very anxious to inject the ducts of the liver in order to ascertain the manner in which they commenced in the lobule, and the precise relation which they bore to the liver cells. This has long been a point of dispute among microscopical observers, and many different and incompatible conclusions have been arrived at by different authorities. In order to prove the point satisfactorily it was obviously necessary to inject the ducts to their minute ramifications, which no one, as far as I was able to ascertain, had succeeded in doing satisfactorily. After death the minute ducts of the liver always contain a little bile. No force which can be employed is sufficient to force this bile through the basement membrane, for it will not permeate it in this direction. When any attempt is made to inject the ducts, the epithelium and mucus, in their interior, form with the bile an insurmountable barrier to the onward course of the injection. Hence it was obviously necessary to remove the bile from the ducts before one could hope to make a successful injection. It occurred to me, that any accumulation of fluid in the smallest branches of the portal vein or in the capillaries, must necessarily compress the ducts and the secreting structure of the liver which fill up the intervals between them. The result of such a pressure would be to drive the bile towards the large ducts and to promote its escape. Tepid water was, therefore, injected into the portal vein. The liver became greatly distended, and bile, with much ductal epithelium, flowed by drops from the divided extremity of the



duct. The bile soon became thinner owing to its dilution with water which permeated the intervening membrane, and entered the ducts. These long narrow highly tortuous channels were thus effectually washed out from the point where they commenced as tubes not more than 1-3000th of an inch in diameter, to their termination in the common duct, and much of the thick layer of epithelium lining their interior was washed out at the same time. The water was removed by placing the liver in cloths with sponges under pressure for twenty-four hours or longer. All the vessels and the duct were then perfectly empty and in a very favourable state for receiving injection. The duct was first injected with a colored material. Freshly precipitated chromate of lead, white lead, vermilion, or other colouring matter may be used, but for many reasons to which I have alluded, the Prussian blue injection is the one best adapted for this purpose. It is the only material which furnishes good results when the injected preparations are required to be submitted to high magnifying powers. Preparations injected in this manner should be examined as transparent objects.\* They may be mounted in the ordinary preservative fluids or in Canada balsam, but glycerine forms the most satisfactory medium for their preservation.

**206. Of Injecting Lymphatic Vessels.**—It is very difficult to find and insert a pipe into a lymphatic vessel. When it is desired to inject these tubes it is usual to inject from the large trunk of the thoracic duct. I have however found that by injecting water into the *blood vessels*, the lymphatics and lacteals become distended by the transudation of the fluid, and in this distended state it is easy to insert the pipe. The pipe having been tied in the vessel, the water is absorbed as described in § 205, and the injection may then be forced in, care being taken to use very gradual pressure, so that the coats of the vessels may be sufficiently stretched to allow the injection to pass between the valves, *without being ruptured*. In this way I have succeeded in injecting the vessels of even a part of one organ. (*"Archives"* Vol. I., p. 113.)

**207. Of Preparing Portions of Injected Preparations for**

\* *"On the Anatomy of the Liver of Man and Vertebrate Animals."*—London: John Churchill, 1856.

**Microscopical Examination.**—When thin tissues, such as the mucous membrane of the intestines or other parts, have been injected, it is necessary to lay them perfectly flat, and wash the mucus and epithelium from the free surface, either by forcing a current of water from the wash-bottle, or by placing them in water and brushing the surface gently with a camel's hair brush. Pieces of a convenient size may then be removed and mounted in solution of naphtha and creosote, in dilute alcohol, in glycerine, or in gelatine and glycerine. The most important points in any such injections are shown if the preparation be dried and mounted in Canada balsam. The specimen must, in the first place, be well washed and floated upon a glass slide with a considerable quantity of water, which must be allowed to flow off the slide very gradually. The specimen may then be allowed to dry under a glass shade, in order that it may be protected from dust. The drying should be effected at the ordinary temperature of the air, but it is much expedited if a shallow basin filled with sulphuric acid be placed with it under the same bell-jar.

Of solid organs, such as the liver and kidney, thin sections as well as portions from the surface should be preserved. Thin sections may be made with the ordinary scalpel or with Valentin's knife, if an extensive one be required. The surfaces of the section should be well washed, and it may then be mounted in one of the methods previously described.

**208. Of the best mode of Destroying the Life of Animals intended for Injection.**—I have tried various plans of destroying the life of animals intended for minute injection, and have found that in death by sudden shock the vessels remain in a relaxed state for a sufficient time after death to enable us to complete the injection. In some cases a good result is gained by destroying life in an atmosphere of carbonic acid, but I find that the very sudden death produced by a fall from a height, dashing on the ground, &c., is the most advantageous. Any small animal may be wrapped up in a cloth and dashed suddenly and with some force against the ground. Care must be taken to avoid rupturing any of the tissues by protecting the animal with several folds of the cloth. Swinging very rapidly through the air also destroys life very suddenly, without causing sudden contraction of the muscles.



Fig. 186.



§ 197.

Fig. 187.



§ 197.

Fig. 188.



§ 196.

Fig. 189.



§ 204.

Fig. 186. Vessels of gall-bladder injected. A double injection of arteries and veins.  
 Fig. 187. Capillaries of the liver injected with chromate of lead (Rainey).  
 Fig. 188. Capillaries injected with Prussian blue fluid.  
 Fig. 189. To illustrate the mode of injecting a piece of intestine.



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## CHAPTER VIII.

OF CHEMICAL ANALYSIS IN MICROSCOPICAL INVESTIGATION.—*Instances of the use of Reagents—Preliminary Operations—Reaction—On Filtering—Evaporation and Drying—Incineration—Apparatus—Chemical Microscope—Examining Substances at a High Temperature. REAGENTS AND THEIR ACTION.—Alcohol—Ether—Effects of Alcohol and Ether—Nitric Acid—Sulphuric Acid—Hydrochloric Acid—Acetic Acid—Chromic Acid—Effects of Acids upon Organic Structures—Solution of Potash—Solution of Soda—Effects of Alkalies upon Organic Structures—Potash and Soda—Ammonia—Nitrate of Barytes—Nitrate of Silver—Oxalate of Ammonia—Iodine Solutions. OF APPLYING TESTS TO MINUTE QUANTITIES OF MATTER.—Bottles with Capillary Orifices for Tests—Capillary Tubes, with India-rubber tied over the Top—Testing for Carbonates, Phosphates, Sulphates and Chlorides. OF OBTAINING CRYSTALLINE SUBSTANCES FROM THE FLUIDS AND TEXTURES OF ORGANISMS.—Formation of Crystals—Influence of various Constituents upon the Crystallization—Separation of Crystals from Non-Crystalline Organic Substances—Examination of Crystals under the Microscope. OF OBTAINING CRYSTALS FOR EXAMINATION.—Preservation of Crystals as Permanent Objects. OF THE HARDENING PROPERTIES OF DIFFERENT CHEMICAL SOLUTIONS.—Mr. Lockhart Clarke's plan of Preparing Thin Sections of the Spinal Cord—Method of rendering Soft Tissues Hard and Transparent.*

OF THE ADVANTAGES OF CHEMICAL REAGENTS IN MICROSCOPICAL INVESTIGATION.

209. *Of Chemical Analysis in Microscopical Investigation.*  
—I have already referred to the influence which the refractive power of the medium in which any structure is immersed

exerts upon its appearance in the microscope. We have now to discuss the advantages derived from the chemical action of certain solutions upon various specimens. This part of the subject is most important, and is perhaps of all the various branches of microscopical research, that from which the greatest advantages may be expected to result. It is an investigation which will certainly reward all who earnestly devote themselves to its study. It is certain that great changes will take place in our views of the nature of many minute structures when chemical analysis shall be more intimately associated with microscopical inquiry. Although by the microscope we can say that such a texture is granular, fibrous, opaque, perfectly clear, &c., we learn in such an examination nothing more of its nature. Since these appearances are manifested by several different materials, it is necessary to resort to a chemical examination to discover the nature of the substance to which the microscopical characters are due. If the composition of any body having well-defined microscopical characters has been once made out, by resorting simply to microscopical examination, we are enabled to recognise it whenever we meet with it afterwards. Some bodies always produce well-recognised crystals when treated with a certain chemical reagent, and we know that although there may be in nature other crystals of a different composition, but of precisely the same form, these latter could not be produced under the same circumstances as the former; hence in such a case we may feel as confident of the nature of the substance as if an ultimate analysis were made of it.

Besides the ordinary uses to which they are applied, chemical reagents are useful in removing certain components of a structure which interfere with the examination of other constituents, in altering the character of certain tissues without dissolving them, as for instance by increasing their transparency or opacity, or in modifying the physical structure of textures in such a manner as to render it more convenient to cut sections or to perform other chemical operations necessary for the demonstration of their structure.

By an acquaintance with the behaviour of certain substances with particular chemical reagents, and the application of this knowledge to microscopical investigation, we are often enabled to distinguish peculiarities of structure, to ascertain the chemical composition of minute quantities of matter, and to demonstrate



clearly the existence of compounds with the greatest certainty, which would entirely escape our observation if we subjected them separately to the most careful chemical analysis, or to the most searching microscopical examination.

The application of chemical analysis to microscopical investigation, and the examination of crystalline forms in the microscope, has thrown a new light upon the nature of many physiological changes which are constantly taking place in living bodies in health, and has enabled us to investigate more satisfactorily the modifications which these processes undergo when influenced by circumstances interfering with or counteracting healthy actions.

**210. Instances of the Use of Reagents.**—As an instance of the great advantage of the application of a few simple tests to microscopical investigation, I may refer to the different effects of ether upon fat globules (which are so commonly found in different tissues) and crystalline bodies composed of phosphate or carbonate of lime, which sometimes resemble them so nearly in refractive properties, in form, and in general appearance, as to have led to mistakes with reference to their nature. The application of a drop of ether has no effect whatever upon the latter, but instantly dissolves the former. Phosphate of lime is readily soluble in dilute acids, while fat is not acted upon by these reagents. Various insoluble saline materials not unfrequently prevent us from seeing the anatomical elements of which a tissue is composed. A knowledge of the nature of these often enables us very easily to remove them. Supposing, for instance, the saline matter consists of carbonates or phosphates of lime or magnesia, we have only to add a drop of dilute acid which dissolves them completely.

The action of acids and alkalies is often very valuable in rendering structures transparent, which are too opaque for examination in the ordinary state. If a portion of tendon, composed of white fibrous tissue (Plate XXVIII, Fig. 112), which is very opaque in its ordinary state, be immersed in acetic acid, or in a dilute solution of potash or soda, it soon becomes clear and transparent, and if the operation be conducted with certain precautions, many of its original characters may be brought back by subsequently neutralizing the acid or alkali.

The cell wall, or rather the outer part of the cell, which is in many cases too opaque to enable us to see the nucleus in the



interior may be made by reagents perfectly transparent so that the nucleus becomes distinct and well defined. This change may be easily effected by either of the reagents alluded to in the last paragraph. Albuminous textures generally may often be rendered very transparent by the action of acetic acid, or by the addition of a drop of dilute caustic potash or soda.

#### CHEMICAL AND MICROSCOPICAL EXAMINATION.

**211. Preliminary Operations.**—In the first place we should note carefully the general characters which the substance exhibits; its form, colour, size, weight, hardness, &c.; and fluidity, transparency, tenacity, &c., in the case of liquids. Portions of solid textures, and the deposit from fluids must be subjected to microscopical examination, but their reaction should be always ascertained in the first instance.

**212. Reaction.**—The reaction of any moist substance is found out by testing it with a piece of blue, and reddened, litmus paper. If the matter be dry, or the reaction of a vapour is to be tested, the paper must be first moistened with a drop of distilled water. The *blue litmus paper* is *reddened* by *acids*, and the *red paper* is turned *blue* by *alkalies*. The reddened litmus paper is prepared by adding a very small quantity of acetic acid to the infusion of litmus into which it is to be dipped.

If an *acid reaction* is due to the presence of carbonic acid, the blue colour will be restored upon gently warming the paper upon a glass slide over a lamp, or upon a warm plate.

An *alkaline reaction* may depend upon the presence of *volatile* or *fixed alkali*. The red colour is restored upon warming the paper which has been rendered blue by the presence of volatile alkali (ammonia or carbonate of ammonia), while it is not restored if the change is produced by the presence of a fixed alkali (potash, soda, or their carbonates, or an alkaline phosphate, &c.).

**213. On Filtering.**—The process of filtration is one which the microscopist as well as the chemist frequently has to perform. To filter a deposit from a solution, in quantity, is easily effected by the use of ordinary filtering paper, folded (Plate XLI, Fig. 190),

Fig. 190.



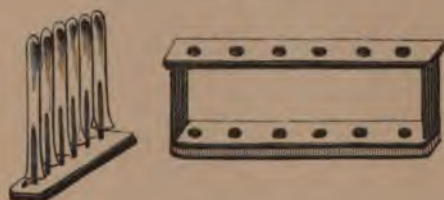
§ 213.

Fig. 191.



§ 213.

Fig. 192.



§ 216.

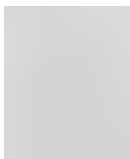
Fig. 193.



§ 216.

- Fig. 190. To illustrate the mode of folding filtering paper.  
 Fig. 191. Funnel arranged for filtering.  
 Fig. 192. Test tubes with stand and drainer.  
 Fig. 193. Box with reagents and other apparatus required by the microscopist.

[To face page 126.]



and placed in a small glass funnel (Fig. 191, Plate XLI). But sometimes we find it necessary in microscopical analysis, to filter the deposit from a single drop of fluid. This may be effected by cutting a very narrow strip of filtering paper, and bending it into a V form, upon one of the glass slides. The drop is made to pass between the limbs of the V, and upon inclining the slide, clear fluid will gradually pass through the apex of the V, and can be conducted away to another part of the slide, by a very fine glass rod, where other tests may be applied.

**214. Evaporation and Drying.**—The evaporation of fluids, and the desiccation of organic solids, must always be conducted over a water-bath, otherwise there is great danger of decomposition occurring. For operations upon small quantities, the water-bath represented in Plate XXI, Fig. 76, will suffice, or the cans of the injecting apparatus, Plate XXXVIII, Fig. 180, may be removed, and basins placed over the holes.

In endeavouring to obtain crystals of organic substances, it is always advantageous to evaporate the solution over the surface of sulphuric acid under a bell-jar, Plate XXIII, Fig. 92, or, what is better still, in vacuo, Plate XXXV, Fig. 160. In some instances, the evaporation may be conducted by simply exposing the liquid placed in a basin or watch-glass, and covered lightly with paper, to the air; or, where very slow evaporation is necessary, the watch-glass may be covered over with a bell-glass.

**215. Incineration.**—By incinerating a small portion of any organic substance, upon a piece of platinum foil, or in a platinum or porcelain crucible, we are enabled to ascertain whether it contains inorganic salts, or consists entirely of organic matter, in which case the substance leaves only a black residue, which burns off entirely after a short time. In order to obtain the inorganic constituents perfectly free from carbon, it is sometimes necessary to keep the mass, for a considerable time, at a dull red heat. The addition of a drop of nitric acid, causes the rapid oxidation of the carbon. If the temperature be too high, the process is often much retarded, in consequence of the fusion of some of the salts, as the phosphates and chlorides, and the inclusion of small masses of carbon, which are thus protected from the action of the atmosphere. The platinum basin or foil



may be supported over the lamp upon a piece of wire, bent in form of a triangle, or upon one of the small rings attached to the spirit lamp (Plate XXI, Figs. 72, 73). It may be removed from the lamp with the aid of an old pair of forceps.

**216. Apparatus.**—The chemical apparatus which is necessary in the course of microscopical investigation is very simple, the greater number of instruments have already been referred to. The following are among the most important pieces of apparatus :—

A few conical glasses of different sizes, § 171. Apparatus for taking specific gravities. Test-tubes of various sizes, arranged on a stand, Plate XLI, Fig. 192. Spirit-lamps, with various supports, Plate XXI, Fig. 73, or, where gas is laid on, the gas-lamp, Plate XVIII, Fig. 66. Glass funnels and filtering paper, and porcelain basins, watch-glasses ; a simple water-bath, Plate XXII, Fig. 76, or the injecting can, Plate XXXVIII, Fig. 180, may be used, if several evaporations are to be conducted at once. A small platinum capsule, a strip of platinum foil, a blow-pipe, pipettes, Plate XXXVII, Fig. 172, and glass stirring rods, a box of reagents in small bottles, Plate XLIII, Fig. 198, and papers, complete the apparatus. All these may be obtained packed in a box of convenient size, Plate XLI, Fig. 193.

**217. Microscope for Examining Substances Immersed in Acids and Corrosive Fluids.**—In examining preparations which require to be immersed in strong acid, in the ordinary microscope, it is not easy to prevent the fumes from injuring the work of the instrument. Considerable inconvenience is experienced in examining fluids while hot, in consequence of vapour which rises, condensing on the object-glass, and rendering the object invisible.

These inconveniences are entirely obviated by the ingenious microscope invented some years ago by Dr. Lawrence Smith, of Louisville, United States (*"American Journal of Science,"* second series, Vol. XIV, 1852). The inverted chemical microscope is represented in Plate XLII, Fig. 194.

By this arrangement the object-glass is always kept perfectly clear, while of course the definition of objects is not in any way interfered with.



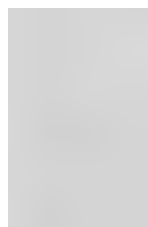
Fig. 194.



§ 217.

The inverted microscope of Dr. Lawrence Smith, of Louisville.

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**218. Of Examining Substances at a High Temperature.—**

By placing a brass plate upon the stage of the instrument just described, and allowing one end to project over the edge, so that it may be conveniently heated by a spirit-lamp, any substance may be kept warm upon a glass-slide, while being subjected to microscopical examination. When a high temperature is necessary, I have adopted the plan represented in Plate XXXV, Fig 158. A square copper tube is arranged to lie flat upon the stage of the ordinary microscope. A spirit-lamp is placed at its lower opening, while the heated air escapes from the upper end. At that part where the glass slide is to be placed, the lower wall of the tube is composed of glass, while at the upper part is an opening, which allows the heated air to come into actual contact with the glass slide.

**REAGENTS AND THEIR ACTION.**

The reagents necessary are not very numerous; they should be perfectly pure. Of the greater number only very little is required; but of alcohol, ether, and one or two others, it is necessary to have a half-a-pint or more. The stock reagents should be kept in stoppered bottles of about the capacity of two ounces.

**219. Alcohol.**—Alcohol of different strengths will be required for the purpose of dissolving certain substances, and for separating them from other constituents, which are insoluble in this reagent. Alcohol should always be diluted with distilled water, and it is better to prepare a considerable quantity at a time. It is convenient to have two or three bottles which will hold about two quarts each. The strength of each should be written upon a label attached to the bottle. The importance of alcohol as a preservative solution has been already referred to in § 99.

**220. Ether, Chloroform.**—An ounce or two of ether will be quite sufficient for microscopical purposes. It should be kept in a stoppered bottle, provided with a glass cap, to prevent loss by evaporation. A little should also be kept in one of the small glass bottles with capillary orifices, § 236, for the convenience of applying to cells containing highly refracting globules, resembling oil, &c., under the microscope.

**221. Effects of Alcohol and Ether.**—Alcohol coagulates albuminous matters. Germinal matter is always rendered granular by this reagent. Many transparent tissues are corrugated, and rendered more or less opaque by alcohol. It dissolves certain forms of fatty matter, resinous materials, many forms of vegetable and animal coloring matter.

Ether is of great use for dissolving various kinds of fatty matter. In many cases (as, for example, in common milk) the oil globule is covered with a caseous or albuminous investment, which protects it from the action of the ether. In this case it is necessary to add a drop of acetic acid, or solution of potash or soda, to dissolve the membrane, when the ether will at once act upon the fat.

Chloroform is a valuable fluid for dissolving Canada balsam. Solutions of this substance in chloroform are preferred by many to ethereal solutions.

**222. Nitric Acid** should be kept of two different degrees of concentration; one the strongest that can be procured, and another containing about twenty per cent. of the strong acid. This last is the acid most used by the microscopist, especially in separating muscular fibre cells. It is prepared by mixing one part of the strong commercial acid with five parts of distilled water.

**223. Sulphuric Acid** is sometimes required undiluted, but a small bottle of diluted acid (one of acid to five of water) should also be at hand. The pure colourless acid should always be procured; it is to be purchased for about 1s. 6d. a pound, but only very small quantities are required.

**224. Hydrochloric Acid** may be obtained perfectly colourless. It may be kept in the pure state and diluted as required.

**225. Acetic Acid.**—Two specimens of acetic acid will be found convenient. One, a solution of the strongest acid which can be procured; the other, containing about twenty per cent. This is prepared by dissolving one part of the strongest liquid acid, or of the pure *glacial acetic acid*, in five of water. The *glacial acetic acid* is now commonly employed for photographic purposes, and can, therefore, be very readily obtained.



**226. Chromic Acid** is usually required very dilute. For the purpose of hardening tissues a watery solution of a straw colour will be found strong enough. It is easily prepared by dissolving a little of the crystallized chromic acid in distilled water.

The crystallized acid may be prepared by decomposing 100 measures of a saturated solution of bichromate of potassa, by the addition of 120 to 150 measures of pure concentrated sulphuric acid. As the mixture becomes cool, crystals of chromic acid are deposited, which should be dried and well pressed on a porous tile, by which means the greater part of the sulphuric acid is removed, and the crystals obtained nearly pure.

**227. Effects of Acids on Organic Structures.**—The effects of the application of cold strong acids to animal textures are very variable; in some instances the tissue is completely destroyed, while in others scarcely any effect seems to be produced. The mineral acids generally coagulate albuminous tissues, and render their microscopical characters confused and indistinct. Tribasic phosphoric acid, however, is an exception to this. Acetic acid dissolves many of the substances allied to albumen. The appearance of some structures is scarcely altered by the application of a strong acid; for instance, the blood corpuscles shrink a little, but exhibit their usual form and general characters for some time after the addition of strong nitric acid, and the cells of the epidermis and nail, although turned of a yellow colour, are not destroyed; the latter are separated somewhat from each other, and their outline is often made beautifully distinct. Most of the mineral constituents of the body, insoluble in water, are directly dissolved by the acids. Strong nitric acid is a very useful reagent for demonstrating vegetable cellular structures.

*Acetic Acid.*—Acetic acid is one of the most useful reagents to the microscopical observer. It has the property of dissolving granular matter composed of albuminous material, and causes cell-wall and many kinds of formed material to become very transparent; although it often renders the nucleus darker and more distinct. In many instances the action of the acid upon the cell wall is curious; the formed material becomes more pulpy and thicker, and approaches more nearly in density and refracting power to the solution in which it is immersed. In numerous instances, by adding a saline solution to cells which have been previously rendered transparent by acetic acid, they



again contract, and the outline becomes distinct. In some cases however, the outer part of the cells is actually dissolved by the acid, and the germinal matter is set free. Acetic acid will be required of various strengths, the most useful proportion being one part of the strong acid to three or five of water. Acetic acid is very frequently used to make epithelial structures transparent, in order that the arrangement of the minute vessels and nerves in papillæ, &c., may be demonstrated, as in the case of the tongue, skin, &c. Sections of preparations which have been hardened by maceration in alcohol, often require boiling slightly in acetic acid before they can be rendered transparent. The action of acetic acid on white fibrous tissue is very characteristic, as it converts it into a transparent jelly-like mass, in which a few nuclei are visible. Upon the yellow element, on the other hand, this reagent exerts no action whatever.

Acetic acid may also be employed for testing crystalline bodies, as phosphates and carbonates. It distinguishes phosphate or carbonate of lime from oxalate of lime (all of which are insoluble in water), by dissolving the two former, while it does not affect the latter even if boiled with it.

The action of acetic acid, upon any particular tissue, upon any form of cells, fibres, &c., that are subjected to examination, should always be specially noted. Many tissues are quite insoluble in acetic acid, though they are not rendered opaque by it.

*Nitric Acid.*—Strong nitric acid dissolves albuminous substances, but first colours them deep yellow. Dilute nitric acid is much employed in microscopical research.—An acid composed of one part of acid to two or three of water, forms a good solution for hardening some structures previous to cutting thin sections. The thin sections may sometimes be rendered very transparent by being treated afterwards with dilute caustic soda. For demonstrating muscular fibre-cells, nitric acid is a valuable reagent. For this purpose the solution should contain about twenty per cent. of strong acid, and the muscular fibre should be allowed to macerate in it for some days, when small pieces may be removed with scissors, and after being carefully torn up with fine needles, subjected to examination.

When we wish to obtain portions of glandular structure isolated from one another, it is a good plan to soak the tissue for some days in dilute nitric acid (one part of acid to six or seven of water), when the areolar tissue becomes softened, and at the

same time the gland structure is rendered more firm, and may be isolated very readily with the aid of needles. In this manner the gastric glands, the secreting follicles of the pancreas, and salivary glands may often be very satisfactorily demonstrated. The so-called fibre cells of organic muscles are to be isolated in the same way.

By boiling animal tissues in strong nitric acid, they become destroyed, while any siliceous constituents remain behind unaltered. In this manner, the siliceous skeletons of the *Diatomaceæ* may be separated from any organic matter with which they may be combined. This is one of the processes employed for obtaining these beautiful objects, from guano.

*Sulphuric Acid.—Hydrochloric Acid.*—The pure concentrated acids only should be used for microscopical investigation. Concentrated sulphuric acid causes epidermic structures to swell up very much, and the cells to separate from each other so as to be readily isolated. Boiling acid completely dissolves them. In the examination of hair, strong sulphuric acid will be found to render the outline of the cells very distinct.

Hydrochloric acid is usually employed for dissolving out the mineral constituents of certain tissues, such as bone or teeth. As a rule, it is better to use dilute acid (one of acid to three or four of water), in which case, however, a longer time must of course be allowed, than when the acid is concentrated.

**228. Solution of Potash** should be kept of two or three different degrees of strength. One, the strongest which can be obtained; another, made by mixing one part of the strong potash with three or four of water; and a solution consisting of one part of liquor potassæ to eight or ten of water will be found of a useful strength for the examination of many preparations.

**229. Solution of Soda** is generally required very dilute. It may be made by mixing one part of the strong solution of the shops with five or six of water; but this, for many purposes, will require to be still further diluted. Or, about twenty-five grains of the fused soda may be dissolved in an ounce of distilled water.

**230. Effects of Alkalies on Organic Structures: Potash and Soda.**—The action of alkalies, even when cold in a very dilute state, is to dissolve most animal textures. Cell-membranes are

frequently almost instantly dissolved, while the nucleus (germinal matter) appears to be altered but slightly.

Alkalies are also employed for dissolving certain crystalline substances which are occasionally found in animal tissues, such, for instance, as the urates.

The action of potash and soda upon animal structures is very similar. Both dissolve substances of an albuminous nature, but the effect of soda is more gradual, and it has been found that for most purposes in microscopical research, this reagent possesses advantages over potash.

The solution of potash is the ordinary *liquor potassæ* of the pharmacopœia, and the solution of soda is prepared in the same manner. These solutions may be diluted with water to the required strength. Potash and soda are employed where a tissue is to be rendered more transparent for the purpose of demonstrating the arrangement of the nerves or other anatomical elements not soluble in this reagent.

These reagents dissolve the layer of epithelium covering mucous membranes, or render it perfectly transparent, so that the arrangement of the structures beneath the basement membrane can be easily demonstrated. In investigating the termination of the nerves and vessels in papillæ and other structures, they are very valuable, especially the latter.

For the purpose above mentioned, the alkalies should be diluted with water. The changes are expedited by the application of heat, which, however, must not be too great, for fear of complete solution taking place. The structure may be heated with the solution in a test tube.

Some animal textures become hardened by prolonged maceration in carbonate of potash, but this plan does not appear to be so generally useful as others previously indicated. Epidermic structures are not much altered by these salts.

The introduction of different chemical solutions by injection, has been discussed in page 112. I strongly recommend this plan of subjecting the tissue to the action of the reagent. See also Chapter X.

**231. Ammonia.**—Solution of ammonia, made by mixing one part of the strongest *liquor ammoniæ* with three of water, will be found sufficiently strong for all the purposes for which this reagent will be required.



**232. Nitrate of Barytes.**—A cold saturated solution of the salt forms a test solution of convenient strength. It should be filtered before use. A solution of nitrate of barytes is employed as a test for sulphuric and phosphoric acids. The precipitated sulphate of baryta being insoluble both in acids and alkalies; while the phosphate of baryta is readily soluble in acids, but insoluble in ammonia.

**233. Nitrate of Silver.**—A solution of nitrate of silver is prepared by dissolving one hundred and twenty grains of the crystallized nitrate in two ounces of distilled water, and filtering if necessary. Nitrate of silver is employed as a test for chlorides and phosphates. The *white* precipitate of chloride of silver is soluble in ammonia, but insoluble in nitric acid. The *yellow* precipitate of tribasic phosphate of silver is soluble in excess of ammonia, as well as in excess of nitric acid.

**234. Oxalate of Ammonia.**—Some crystals may be dissolved in distilled water, and, after allowing time for the solution to become saturated, it may be filtered. Oxalate of ammonia is used as a test for salts of lime. Oxalate of lime is insoluble in alkalies and in acetic acid, but soluble in the strong mineral acids. In testing an insoluble deposit for lime, it may be dissolved in nitric acid and excess of ammonia added; the flocculent precipitate is readily dissolved by excess of acetic acid, and to this solution the oxalate of ammonia may be added. The precipitation of oxalate of lime is favoured by the application of heat. Many deposits of phosphate are with great difficulty soluble in acetic acid, hence the necessity of first adding nitric acid, as above directed.

**235. Iodine Solutions.**—An aqueous solution is easily prepared, by dissolving a few grains of iodine in some distilled water, until it acquires a brownish-yellow colour. A solution of iodine is sometimes useful for colouring certain animal and vegetable textures, which are so transparent as to be scarcely distinguishable upon microscopical examination. In the examination of many such structures, great assistance will be obtained from the use of coloured solutions; for delicate textures, like the cell wall and basement membrane, &c., can be far better distinguished when a faint tint is communicated to them, than when perfectly



colourless. When a membrane is to be made more distinct, it may be immersed in a little Prussian blue fluid (§ 196), the minute particles of which adhere to it, and enable us to trace its outline clearly, or in a weak solution of magenta.

Iodine is principally employed as a test for starch which is rendered blue by an aqueous solution, even when very dilute. Albuminous matters and tissues are coloured yellow by iodine, and vegetable cellulose also receives a brownish-yellow tinge. The addition of sulphuric acid (one part of the strong acid, two parts of water) to albuminous matter stained with iodine, causes no change, but cellulose under the same circumstances becomes blue. In cases where substances allied to starch and cellulose (amyloid matters) are found associated with the albuminous matters, a purple, bluish, or greenish tinge results from the action of iodine and sulphuric acid.

A strong solution of iodine may be obtained by employing a solution of iodide of potassium to dissolve the iodine (one grain of iodine and three grains of iodide of potassium, to one ounce of distilled water).

Schultz recommends the following iodine solution. Zinc is dissolved in hydrochloric acid;—the solution is permitted to evaporate in contact with metallic zinc until it attains the thickness of a syrup; and the syrup is then saturated with iodide of potassium. The iodine is next added, and the solution, if necessary, is diluted with water. Professor Busk gives the following directions for preparing this solution: one ounce of fused chloride of zinc is to be dissolved in about half an ounce of water, and to the solution (which amounts to about an ounce fluid measure), three grains of iodine dissolved, with the aid of six grains of iodide of potassium, in the smallest possible quantity of water, are to be added (*"Trans. Mic. Soc.,"* Vol. I., p. 67). I have employed a solution prepared in this manner, and can speak very highly of its utility. In making it, it is necessary not to fuse the chloride of zinc much, or to use a very high temperature, as decomposition is very apt to take place. In testing starch with this solution, it is advisable to add a very little water, as the solution frequently will not act in its concentrated form.

## OF APPLYING TESTS TO MINUTE QUANTITIES OF MATTER.

**236. Bottles with Capillary Orifices for Tests.**—The above tests may be preserved in ordinary stoppered bottles, but I much prefer to keep them in small tubes with capillary orifices, from which only a drop, or a part of a drop, can be expelled when required. Several years since, I arranged all the ordinary tests I required for microscopical purposes in small bulbs which were drawn off to a capillary point. They were provided with glass and gutta percha caps. These bulbs, however, were somewhat inconvenient in consequence of not being made to stand upright, and Mr. Highley substituted for them tubes with flat bottoms and ground glass caps (Plate XLIII, Figs. 195, 197). To fill these bottles I proceed as follows:—A little of the solution is poured into a small basin, the tube being inverted so that its orifice dips beneath the surface of the fluid. Heat being now applied to the body of the bulb, the air in its interior is expanded and partially expelled. As the bottle becomes cool, a certain quantity of the fluid rises up into its interior. Usually, however, it is not possible to introduce more than a few drops in this manner. The bottle is then removed and heated over the spirit-lamp until the drop of fluid in its interior is in a state of ebullition. While the steam is issuing violently from the orifice, I carefully plunge it again beneath the surface of the fluid. As the steam within condenses, the solution rises up in the interior, and would completely fill the little bottle if it were maintained in this position, but when it is about three parts full I remove it from the fluid. If I were to fill it completely it would be difficult to expel the fluid when required. A certain quantity of air, therefore, is allowed to remain within the bottle, and being expanded by the warmth of the hand, the quantity of fluid required can be driven out at pleasure. In this manner fluid may be introduced.

Mr. Highley has made a further modification by arranging the capillary neck in the form of a tubulated stopper, by the removal of which, fluid can be introduced as in filling an ordinary bottle (Fig. 197).

For microscopical purposes bottles with capillary orifices possess many advantages over the ordinary stoppered bottle.

In the *first* place, a most minute quantity of the test can be

obtained without difficulty, and there is no chance of too much escaping.

*Secondly*, there is no danger of the reagent becoming spoilt by the introduction of various substances from without. If an ordinary stoppered bottle be used, a drop of the fluid must be removed with a pipette or stirring rod, but if these should not be quite clean, foreign substances may be introduced, and the reagent spoilt for further operations. Carelessness upon this head will lead to the greatest inconvenience, and give rise to serious mistakes.

*Thirdly*. Testing by means of these little bottles can be conducted in a very short space of time, and they possess the advantage of being packed in small compass. (Plate XLIII, Fig. 198.)

**237. Capillary Tubes with India-rubber tied over the Top.**—Dr. Lawrence Smith, of Louisiana, recommends that the tests should be kept in bottles of two ounce capacity, and instead of a stopper, he inserts a tube in the form of a pipette, the upper open end being covered with a piece of vulcanised India-rubber (Fig 199). By pressing this while the lower end is beneath the fluid, a portion of the air is of course driven out, and a little fluid rushes in to supply its place as soon as the pressure is removed. The tube may then be removed from the bottle, and by again pressing the India-rubber, a drop, or a portion of a drop, is very readily expelled.

**238. Method of Applying Tests to Substances intended for Microscopical Examination.**—The matter to be tested may be placed upon a glass slide, and, if necessary, a drop of water added, to moisten or dissolve it, as the case may be.

In these operations we usually require only a small drop of a solution, and it will be found most convenient, in applying it to the object, to take a drop from the bottle by dipping a stirring-rod into it, and withdrawing it immediately. Enough will be found adhering to the stirring-rod for the purpose required. The rod should not be dipped in a second time, without being first well washed in water,—for if this be not scrupulously attended to, there is great danger of conveying some of the substance intended for examination into the test bottle, in which case the whole contents would be spoiled. Without great care in all our manipulations, there will be much danger of removing a portion



Fig. 195.



§ 236.

Fig. 196.



§ 236.

Fig. 197.



§ 236.

Fig. 198.



§ 236.

Fig. 200\*.



§ 244.

Fig. 199.



§ 237.

Fig. 200.



§ 244.

Figs. 195, 196. Small capillary tube and bulb with capillary orifices and caps, for microscopical testing. Fig. 197. The same, but arranged with a tubular stopper for greater facility of introducing the test solution. Fig. 198. Box with twelve test bottles. Fig. 199. Tube with India-rubber, *a*, tied over upper extremity to remove small quantities of test solution from bottles. It is inserted like a stopper, and ground at the point *b* to fit the neck of the bottle. Fig. 200. To illustrate the appearance of crystals (creatine) under the microscope. Fig. 200\*. Crystals of common salt.

[To face page 138.]





of one substance from a glass slide and carrying it to a deposit which is subsequently examined ;—a result which might lead to great inconvenience and very serious mistakes. Accidents of this kind can always be avoided, by not allowing the drop of the reagent to touch the deposit until the rod has been removed. This can be effected by placing the drop near the substance intended for examination, and then allowing it to come into contact with it, either by inclining the glass slide, or by leading it with a glass rod, to the matter to be tested. Without the greatest attention to cleanliness, the microscopical observer will be constantly led into error, and thereby bring discredit upon himself and upon the science. Nothing is more common than to find a specimen which we are examining in the microscope covered with a vast number of starch granules, which have been introduced from without. Usually they are derived from the squares of thin glass which are commonly kept in a little starch powder to prevent fracture. An intimate friend showed me one day some microscopic preparations which contained bodies of the nature and origin of which he was not aware. Upon examining the slide, I found a number of scales from the wing of a moth, which had no doubt been floating about in the air and had fallen upon the preparations. For all such delicate operations, the specimens should be carefully protected by glass shades.

**239. Testing for Carbonate and Phosphate of Lime, Phosphate of Ammonia-and-Magnesia, Sulphates and Chlorides.**—Now, suppose the nature of the substances composing certain forms of earthy matter is to be ascertained. A small portion, about the size of a pin's head, is placed upon the slide, and covered lightly with a piece of thin glass. Next, a drop of *nitric acid* is expelled (by warming in the hand the bottle with the capillary neck) near to the thin glass. The acid soon reaches the sediment, and the disengagement of a few bubbles of gas may be observed. These are, as it were, temporarily pent up by the thin glass, and prevented from escaping. If there should be any doubt of the action of the acid, we may resort to examination in the microscope, when, if there be very few bubbles, they may be detected. The escape of bubbles of gas indicates the presence of *carbonates*.

The acid solution may be neutralized with *ammonia*. A faint flocculent precipitate may be produced. After this has stood

still for a few minutes it is covered with thin glass and examined under the microscope. It may consist of amorphous granules (Phosphate of Lime) and small crystals, which, if allowed to stand long enough, will take the form of triangular prisms (Phosphate of Ammonia and Magnesia).

If we wish to ascertain the presence of sulphates, a little of the nitric acid solution is treated with *nitrate of Barytes*. An amorphous precipitate of Sulphate of Baryta, insoluble in strong acid and alkalies, takes place, if sulphuric acid be present. The presence of chlorides is detected by the addition of a little *nitrate of silver* to a drop of the solution of the deposit in weak nitric acid. The white precipitate of chloride of silver is insoluble in *nitric acid*, but it is dissolved by *ammonia*.

These will serve as examples of the method of detecting the presence of different substances in a very minute quantity of matter. The indications obtained in this manner are quite as valuable, and may be relied upon with as much certainty, as if we were provided with a very large quantity of material to work upon; in a single drop of a composite solution, the presence of several different acids and bases may be detected.

#### OF OBTAINING CRYSTALLINE SUBSTANCES FROM THE FLUIDS AND TEXTURES OF ORGANISMS.

Under this head it is proposed to give a sketch of a few of the simplest plans of obtaining various crystalline bodies from animal solids and fluids.

**240. Formation of Crystals.**—Some crystalline bodies are deposited from their solution in animal fluids by simple evaporation; others, less soluble, may be deposited by allowing the fluid to stand still for a short time, when certain changes occur in some of its constituents, which lead to the precipitation of some bodies in a crystalline form, such, for instance, as uric acid, or crystals of triple phosphate. In other cases it becomes necessary to add some reagent before the crystals are thrown down, while not unfrequently a long and often complicated chemical analysis is required, in order to isolate some of the substances which were previously held in solution, and obtain them in a crystalline state. The addition of water in some cases causes the most rapid

crystallization, especially when the crystallizable material is contained in a cell, as when water is added to blood, in order to obtain blood crystals. Instead of water, in other instances, it becomes necessary to add alcohol, in which fluid the crystals may be much less soluble than in water.

Crystalline substances which are dissolved in animal fluids, may often be separated in a perfectly pure state by the addition of another fluid in which they are not so readily soluble. This last should be added very gradually, to allow time for the formation of the crystals, otherwise an amorphous precipitate alone results. Many organic substances soluble in alcohol, may be crystallized by the addition of ether, while some are precipitated from their solution in water, by the gradual addition of alcohol.

**241. Influence of various Constituents upon the Crystallization.**—In many instances, it is exceedingly difficult to separate some crystalline bodies from other constituents with which they are retained in solution. In consequence, their solubility is much increased, and their crystallization often prevented. The extractive matters of blood, urine, &c., exert this influence in a marked degree, and it is only of late years that several new bodies of definite chemical composition have been isolated. Creatine and creatinine may be instanced amongst the number, for these were not very long ago included under the indefinite term "extractives." Certain colouring matters of definite composition have also been separated, and it is very probable that as the methods of analysis at our disposal become improved, many new crystalline bodies will be isolated from the extractive matters. A very small quantity of extractive matter entirely prevents the crystallization of urea, while the presence of chloride of sodium favours the separation of this material by forming with it a compound which readily crystallizes in large octohedral crystals even in the presence of extractive matters. The existence of carbonic acid in excess may cause carbonate of lime, triple phosphate, and other salts, to be held in solution. Excess of alkali prevents the precipitation of uric acid, and excess of acid, that of phosphate of lime. Fatty matters dissolve cholesterine, and serum possesses the power of retaining small quantities of both the latter substances in solution. Some crystalline bodies which are soluble at the temperature of the body, crystallize



when the solutions containing them are cooled thirty or forty degrees. The effect of dilution upon retaining crystals in solution, need scarcely be alluded to. Hence, before the presence of many substances can be detected by microscopic examination, certain chemical operations are required in order to separate them from their combinations in the animal body, or for the removal of other substances which interfere with their crystallization.

**242. Separation of Crystals from Animal Substances.**—From what was stated in the last section, it follows that in many instances this is a matter of some difficulty. Not unfrequently, even after crystals have been obtained, if not very soon separated from the fluid in which they were formed, they again undergo solution or become decomposed. If the crystals are not very soluble, the supernatant fluid, or mother-liquor, may be poured off,—the crystalline deposit washed with ice-cold water, and subsequently dried on filtering paper over sulphuric acid, without the application of heat.

If the crystals will not bear the application of water, as much of the fluid as possible must be poured off, and the remainder absorbed with bibulous paper, or they may be placed upon a porous tile, and dried over sulphuric acid in vacuo. In many instances we are enabled to wash the crystals with water, holding a little acid or alkali, or some alkaline salt, in solution, or with alcohol, ether, or some other fluid in which we know them to be quite insoluble.

In cases in which crystals insoluble in water are deposited in animal solids, they may be separated by agitation, when, being heavier than the water, they subside to the bottom, and the lighter animal matter may be removed by forceps, or if in a very minute state of division, poured off with the supernatant fluid. In other cases it may be separated by straining, while the crystals are washed through muslin.

**243. Examination of Crystals under the Microscope.**—Some crystals which have been entirely separated from the fluid in which they were originally deposited, may be examined in the dry way, in water, or other fluid in which they are known to be insoluble, or in Canada balsam; but, as a general rule, it is necessary to examine the crystals as they lie in some of the fluid

in which they have been formed. When they have been obtained by allowing a concentrated solution to cool, some of the inspissated fluid must be removed with the crystals, placed upon a glass slide or in a thin glass cell, covered with a piece of thin glass, and examined in the usual way—first using a low power (an inch), and afterwards a higher power (a quarter), because, although some of the crystals are of a large size, others amongst them, the form of which is very perfect, are often exceedingly minute. The crystals and mother-liquor should not be exposed to the air previous to examination, for in many instances water is absorbed, and partial solution takes place.

**244. Of obtaining Crystals for Examination.**—In order to accustom himself to the necessary manipulation required in the process, the student may evaporate a solution of common salt upon a glass slide, and when it has become sufficiently concentrated it may be covered with a small piece of thin glass, and allowed to cool. When cold it may be subjected to microscopical examination, and beautiful cubes of chloride of sodium will be observed (Plate XLIII, Fig. 200\*). Crystals of several salts may be made in the same simple manner, and from an attentive examination of them, much may be learned. Phosphate of soda, phosphates of soda and ammonia, sulphates of potash and soda, muriate of ammonia, and a variety of other salts, can be readily obtained in microscopical crystals in this manner. Mr. Glaisher has made some beautiful observations on snow-flakes. Copies of his drawings are presented in Plate XLIV, Figs. 201 to 213.

Different faces of the crystal, as it lies in the liquid, may be brought into view by slightly moving the thin glass cover with a fine-pointed instrument, such as a needle, while the preparation is in the field of the microscope. With a little practice, crystals may in this manner be made to rotate in the mother-liquor. Crystals which are precipitated by the addition of some reagent, such as nitrate of urea by nitric acid, must be examined in a little of the solution. The addition of water would, in many instances, destroy them immediately. Beautiful crystals are represented in Plates XXXI and XLIII.

The influence of the crystals upon polarized light (§155) should be examined, and in cases in which the nature of the crystal has not been ascertained, its angles should be carefully measured, and accurate drawings made (§ 68). Their behaviour with chemical

reagents is next to be ascertained, and their solubility in water, alcohol, and other fluids must be noted. For these experiments different portions must be taken and separately tested in the manner referred to in §§ 238, 239.

A drop of the solution may also be evaporated rapidly nearly to dryness, and allowed to crystallize upon the slide without being covered over, when the substance will often be found to assume a variety of beautiful forms, such as crosslets, dendritic expansions, &c., which vary according to the rapidity with which the evaporation has been conducted, and other circumstances.

**245. Preservation of Crystals as Permanent Objects.**—The preservation of the more soluble crystals is attended with the greatest difficulty, except when dried, in which state their characters under the microscope are not well defined. Crystals which very readily deliquesce on exposure to air, must be dried in vacuo, removed quickly to a cell, the cover of which must be firmly cemented down at once. Some crystals may, however, be dried and mounted in Canada balsam; others, such as oxalate of lime, cystine, triple phosphate, &c., can be well preserved in aqueous solutions, containing a little acid in the case of the two former substances, or an ammoniacal salt, in the latter instance, in which the crystals are known to be insoluble. Crystals which contain water of crystallization must be preserved in a drop of the mother-liquor; but in many instances they alter much in form, and when we come to examine them, instead of finding a great number of small, well-formed crystals, as when the preparation was first put up, nothing remains but one or two large ill-shaped ones. The concentrated mother liquor often acts upon the cement with which the glass cover is fixed on the cell, and very soon air enters, and the preparation is destroyed. Many crystals may be preserved in strong glycerine without much change taking place. I have some crystals of Guinea-pig's blood which have been preserved for upwards of three years in this medium.

#### OF THE HARDENING EFFECTS OF CHEMICAL SOLUTIONS.

**246. Of the Hardening Properties of Different Chemical Solutions.**—The consistence of many tissues is so soft that it is



Figs. 201-219.

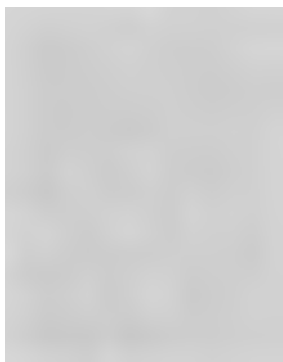


§ 243.

Various forms of snow crystals, drawn by Mr. Glaisher in the winter of 1855.  
 Mic. Journ., Vol. III, p. 179.

[To face page 144.]





absolutely impossible to obtain a thin section ; while, by tearing off a small piece, the relations of the component parts is usually so much altered, as to render the specimen useless for the purpose of examination. In this case our only chance is to harden the texture by some reagent in such a manner that, although its microscopical characters are not altered, a thin section may be readily obtained.

It is even possible in some instances to render a soft tissue sufficiently hard to enable us to cut very thin sections, which may afterwards be restored to their former consistence by the complete removal of the hardening solution.

The nature of the solution employed for hardening a tissue will depend upon the character of the texture itself. Many tissues may be hardened in alcohol, others may with advantage be soaked in a weak solution of chromic acid, which contains a sufficient quantity of the solid acid to render the solution of a pale straw-colour. Various saline solutions are also sometimes employed for hardening tissues, but in consequence of the alteration they produce in the texture of the substance, they are not well adapted for many microscopical specimens. Boiling in water, is often resorted to for the same purpose. In this way very thin sections of such textures as muscular fibre may be obtained which may afterwards be rendered transparent by being soaked in syrup or glycerine, or by the addition of a little solution of caustic soda or potash ; nitric acid (§ 227), and a solution of sesquichloride of iron are also employed for hardening some tissues with advantage.

The hardening properties of the solutions which I have just referred to, depend essentially upon their power of coagulating albuminous substances, and in the majority of instances the coagulation is associated with a certain opacity quite incompatible with the satisfactory examination of the tissue by transmitted light, and as I have before hinted, it is absolutely necessary to render such a specimen transparent after the thin section has been obtained. It is well to bear in mind that before we can submit many soft structures to microscopical examination, we have to consider what chemical substances are likely to harden them in the most advantageous manner for cutting thin sections, and if by this process the section be made opaque we have next to consider further how its natural transparency may be restored. The chemical nature of the substance to be

examined, its physical properties generally, its refractive power, and its chemical composition, are points which it is most desirable that every microscopic observer should be acquainted with before he commences any particular investigation.

**247. Mr. Lockhart Clarke's Plan of Preparing Thin Sections of the Cord.**—By a peculiar method of preparation my friend Mr. Lockhart Clarke has obtained most beautiful sections showing the arrangement of the nerve-fibres, and vesicles in the spinal cord and other parts of the nervous system. The results of his observations are recorded in the "*Philosophical Transactions for 1851*," Part II. The cord is first hardened in acetic acid and alcohol, when excessively thin sections may be readily obtained with a sharp knife. These are then soaked in pure spirit which permeates the texture in every part, and drives out the acetic acid. It is then transferred to turpentine which expels the spirit, and lastly the sections are mounted in Canada balsam. Transverse and longitudinal sections of the spinal cord prepared by this process are represented in Plate XLV.

**248. Method of Rendering Tissues Hard and Transparent.**  
—I have succeeded in rendering the tissues of the embryos of mammalian animals so transparent that the smallest ossific points can be seen in the temporary cartilages. To dissect these bony points at so early a period, would be a work of immense labour, but by merely soaking the whole organism in the solution all these points become beautifully distinct. The specimen is to be immersed in alcohol to which a few drops of solution of soda have been added, and allowed to remain in it for a few days. When the action has taken place, it is to be removed and preserved permanently in weak spirit. I have a beautiful preparation of this kind which has been kept for nearly ten years, and still preserves its character unaltered. The principle of the action of the fluid may be explained thus, alcohol alone tends to coagulate albuminous textures and render them opaque, at the same time that it hardens them. The alkali, on the other hand, will render the tissues soft and transparent, and, if time were allowed, would cause their complete solution. These two fluids in conjunction harden the texture and at the same time make it clear and transparent. Many soft tissues may thus be hardened sufficiently to enable us to cut very thin sections.

Fig. 214.



§ 247.

Fig. 215.



§ 247.

TO ILLUSTRATE MR. LOCKHART CLARKE'S METHOD OF MAKING PREPARATIONS OF THE SPINAL CORD.

Fig. 214. Longitudinal section through the spinal cord in the neck of a cat. pc. Posterior white column. ac. Anterior white column. g. Grey substance between the white columns. p. Posterior roots of the nerves, consisting of three kinds—*a*, *b*, and *c*. a. Anterior roots of the nerves. ac. A portion of the anterior column, showing the arrangement of the longitudinal fibres.

Fig. 215. A transverse section through one-half of the lumbar enlargement of the cord, representing the course of the fibres of the roots of the nerves, and the transverse commissures through the grey substance. The vesicles have been omitted to prevent confusion. The fibres of the anterior roots, *i*, *i*, on reaching the grey substance, are seen to diverge and cross each other; and those of the posterior roots are seen intersecting each other in every direction.

[To face page 146.]





Preparations of this kind show how much may be effected by the use of very ordinary chemical reagents. By this simple process, a minute dissection which would extend over days is avoided, the chance of losing some of the small ossific points is prevented, while the structures are displayed far more distinctly than could be done by the most careful dissection.

Doubtless there are many other fluids yet to be applied to the purposes of investigation of much greater value than the present one, and I strongly recommend observers to take up this branch of inquiry and endeavour to discover new modes of preparing textures which shall render their minute structure clearly demonstrable.

**249. New Method of Microscopical Analysis.**—Since I have been in the habit of using glycerine as the basis of all my injecting fluids and preservative solutions, I have employed it as the solvent of all tests with the greatest advantages. The reactions are of course slower, but much more perfect. Crystals can be obtained most readily by this process, and as the viscid solutions mix very slowly, most perfect crystals of substances which crystallize with great difficulty in water can be readily obtained. If glycerine be added gradually to many solutions of crystalline matter, crystals are deposited. The various tests may be prepared as usual; Price's glycerine being used instead of water. The iodine reactions can often be obtained most satisfactorily by this mode of proceeding. The solutions may be kept in the little glass bottles described in § 236. Very strong solutions of the nitric and sulphuric acids cannot be obtained in glycerine, but it is seldom that a stronger solution than one part of acid to five of glycerine is required. If a very strong viscid solution of acetic acid be required, the acid may be warmed with lump sugar in sufficient quantity to make a fluid of the consistence of syrup. Glycerine becomes almost the universal medium for the examination, preservation, and qualitative analysis of microscopic objects. It need scarcely be said that glycerine and syrup are miscible in all proportion so that the viscosity of any fluid can be readily increased by the addition of sugar to it. *See Chapter X.*



## CHAPTER IX.

## ON TAKING PHOTOGRAPHS OF MICROSCOPIC OBJECTS.—

*History.* APPARATUS.—*Camera, with Object-glasses and Stage adapted to it—Mr. Wenham's arrangement without a Camera—Camera applied to the ordinary Microscope—Dr. Maddox's Camera—Arrangement of Drs. Abercrombie and Wilson.* ILLUMINATION.—*Sunlight—Artificial Light—Of Focussing—Of the Object-glasses—Stereoscopic Photographs.* CHEMICAL SOLUTIONS.—*Collodion—Nitrate Bath—Of the Developing Solutions—The Fixing Solutions.* PRACTICAL MANIPULATION.—*Cleaning the Glass Plates—Arranging the Camera—Inserting the Plate—Developing the Image—Varnishing the Plate—Of cleaning Old Plates—Of increasing the Intensity of the Negative.* OF PRINTING.—*Preparing the Paper and Printing—Toning Solution—Another Toning Solution—Fixing Solution—Of Mounting the Prints—Photographs of Microscopic Objects for the Magic Lantern.*

It seemed to be a matter of the utmost importance to enter more fully into the subject of photography as applied to the microscope than in the last edition of this work, and as I have not had much practical experience in this department, I have sought the assistance of my friend, Dr. Maddox, to whom I am indebted for all the valuable and practical details that will be found in this Chapter. It need scarcely be said, that success in this most beautiful mode of delineating objects depends entirely upon carrying out practical details, and although the general reader may on this account not take much interest in this Chapter, I shall offer no excuses for devoting so many pages to the subject.

A brief history of the application of photography to copying microscopical objects will be given in the first place, and a short list of reference will be found at the end of the Chapter.



**250. History of the Application of Photography to the Microscope.**—Mr. Dancer, about 1840, produced photographs of microscopic objects by the *gas microscope*, the images being taken upon silvered plates, also images of sections of wood, fossils, &c., were reproduced on paper and glass plates by means of the solar microscope. In 1841, Mr. Richard Hodgson obtained excellent daguerreotypes of microscopic objects. The Rev. Messrs. Reade and Kingsley were early authorities in the employment of photography in this manner; also Mr. Talbot. Dr. Donné, of Paris, about the year 1844, or earlier, issued a work on some of the branches of microscopic anatomy, in which the engravings were produced from plates prepared as daguerreotype plates, sensitized, exposed, developed, and afterwards chemically etched. The delicacy of some of these engravings was very marked. The plates, however, only permitted comparatively few impressions to be struck off before giving evidence of injury.

In October, 1852, we find a paper by Mr. Joseph Delves was presented to the Microscopical Society of London, and in the following number of the "*Quarterly Journal of Microscopical Science*," some beautiful specimens of prints from Mr. Delves' collodion negatives were issued by the then publisher, Mr. Highley. This was one of the earliest publications with photographic illustrations of microscopic specimens. In the same Journal is a valuable contribution by Mr. G. Shadbolt. Since that period the employment of photography in this way has become more general; doubtless many have been occupied with it whose names are not familiar to us. In Paris M. Nachet and M. Bertsch have obtained excellent results. In Germany, Gerlach, Kolmann of Munich, and many others have illustrated memoirs with photographic plates. Sir D. Brewster, in his article "Microscope," "*Encyclopædia Britannica*," last edition, speaks very highly of some photomicrographs exhibited at the Academy of Sciences, Paris, in 1857, by M. Bertsch, the focal length of the objective used being half a millimetre. The objects, a diatom from guano magnified 500 diam.; two specimens of navicula, one  $\times 800$ , the other  $\times 500$ , the field being rendered nearly dark by oblique illumination; human blood globules  $\times 500$ ; and two pictures of salicine, one taken by polarized light. M. Hartnach, Sir D. Brewster says, has constructed a complete instrument for M. Bertsch, the range being from 50 to 1,000 diameters, and from 50 to 150 diameters for opaque objects. The extreme detail,

beauty of texture, and sharp delineation of the objects in the prints from Mr. Delves' negatives marked a very important step.

Within the last few months Dr. Maddox has succeeded in producing some very beautiful photographs, several of which he has placed at my disposal for the illustration of this work. My friend has with much labour produced the very satisfactory photographs in the frontispiece. He tells me in a note that these were obtained in the following manner:—

"Prints selected from some of my negatives, representing objects magnified in various degrees, varying from the  $1\frac{1}{2}$  inch objective to the  $\frac{1}{32}$ th, were placed on a card in such a manner as to try to balance each other in their effects, and such size of card adopted that, when reduced *one half*, it might correspond with the dimensions chosen by yourself for the plate. The card of prints being placed at the requisite distance, a Ross' 15-inch focus landscape lens was used to obtain the negative copies.

"To render the minutest lines, especially in the *Pleurosigma Angulatum*, well evident in the negative, it was necessary not to carry the development or intensifying process too far, or these became filled up and much obscured, hence the interspaces between the figures allowed a little light to pass; as this seemed detrimental and rendered the figures less effective in appearance, these parts have been painted out.

"The illustrations were photographed with the objective stated in the "explanation." The  $\frac{1}{32}$ th objective was made by Mr. Wenham, and through his liberality placed at my service."

Many of these photographs require the magnifying glass to bring out their detail.

My friend Dr. Dean, of Boston, U. S., has just sent me some very perfect photographs of sections of the medulla oblongata. These are by far the most perfect photographs of structures from the higher animals that I have seen. ("The gray substance of the Medulla oblongata and Trapezium," by John Dean, M.D., "*Smithsonian Contributions to Knowledge*," 173.—Washington, 1864).

Many anatomical specimens, however, cannot be copied by photography, especially if they be very thick. The yellow colour of the tissue in most instances precludes the possibility of making a photograph of it, as the transmission of the light is so much interfered with; and this is an especial objection in the case of injections viewed as transparent objects, for the tissue intervening

between the vessels is often so yellow that these intervals in the photograph become as dark as the vessels themselves. My friend Mr. Julius Pollock has nevertheless succeeded in obtaining for me some very tolerable copies of injections of the distribution of the ducts in the liver. By practice, doubtless, many improvements in the process of taking photographs of microscopic objects would be effected.

When only few copies of a work are required, the researches may be very cheaply illustrated by taking photographs of drawings. A large drawing of the object must first be made in the manner described in § 45. From this a negative reduced to the proper size is taken, from which any number of copies may be obtained. In this manner I have illustrated my memoir on the anatomy of the liver with upwards of sixty illustrations. (*"The Anatomy of the Liver, 1856."*) The results were not so satisfactory as they might have been, but as all the prints were prepared at home with very limited appliances, very good prints could not be looked for. When many copies of a work are likely to be required, this mode of illustration is not applicable, as the original cost of engraving would soon be covered; but when only a few copies of a great number of drawings are wanted, this plan possesses decided advantages.

Different modes of illumination have been employed. Mr. Delves had used sunlight. Mr. Shadbolt, in 1852, tried some experiments with artificial light, and used with great success a small camphine lamp. Mr. G. Busk employed gaslight from an argand burner in 1854; and in November of the same year Mr. Wenham states that, although with the use of camphine and gas light he was dissatisfied, yet the succession of electric sparks (about 100), from a small Leyden jar of 30 inches coated surface, gave actinic rays of sufficient intensity to produce a good impression on a sensitive collodion plate. Mr. Wenham, however, upon the whole gave the preference to sunlight. Mr. Howlett also used sunlight, and condensed it from a plane mirror or solar reflector by a six-inch double convex lens. The Rev. Mr. Kingsley with a special apparatus used the hydro-oxygen light and a screen of esculine. Mr. Bockett in 1862 tried diffused daylight, allowing in some cases an exposure of from 4 to 8 minutes. Dr. Maddox has recently succeeded, by using the brilliant light emitted on the combustion of magnesium wire ( $1\frac{1}{4}$ -inch) held in the flame of a small spirit lamp, and condensed by an ordinary condensing lens.



## APPARATUS.

Two modes of arranging the apparatus have been devised. In the first, the ordinary compound microscope is placed horizontally in connection with an ordinary camera by inserting the eye-piece end (the eye-piece being removed) into the brass setting of a well-made portrait combination (the lenses having been removed), and the aperture around the body of the microscope perfectly closed by any simple method, as a card cap or cone of black cloth or velvet attached to both.

In the second, the ordinary microscope is dispensed with, the objective stage and mirror being adapted to the front of a well-made camera in the place of the usual combination; proper arrangements being made for holding the object, supporting the mirror, and adjusting the different special parts. The pocket-microscope described in § 21 may be adapted to the camera.

**251. Camera with Object-Glasses and Stage adapted to it.**—The apparatus used by Mr. Delves was brought before the public by Mr. Highley, and very much perfected by him. This form of apparatus attracted considerable attention at the late International Exhibition. M. Duboscq also exhibited this arrangement. It seems to meet most requirements for moderate distances, but demands especial outlay. Mr. Highley has lately introduced further improvements, which makes his apparatus still more perfect. (*See Plate XLVI*).

**252. Mr. Wenham's Arrangements without a Camera.**—Mr. Wenham dispenses with the use of the ordinary camera, and yet attains its purpose most completely with sundry advantages. He advises a room to be selected having a window or aperture with free access to sunlight. This is closed by a shutter having a hole about 3 inches in diameter; upon the outside of this aperture is arranged a solar reflector or plane mirror in such a manner as to be capable of being worked round its centre at the necessary angle, on the outside, by passing the hand through another hole in the shutter to the margin of which a flexible sleeve is attached. The microscope body is arranged horizontally on a table or bench, so that its axis corresponds to the centre of the aperture. The stage with the object slide clamped on it in proper position, is placed near this aperture on the inside, the light around the stage

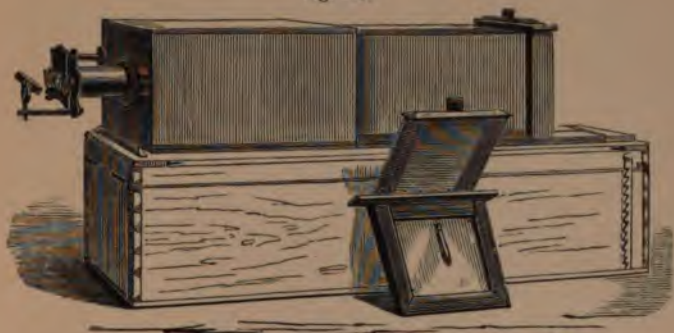


being shut off by a piece of black cloth. On the bench a vertical stand, consisting of a board with a heavy base, is placed at any desirable distance from the eye-end of the microscope; this board is supplied with two "under-cut fillets" to hold the sensitized plate when ready. The mirror is first properly arranged so as to throw an equal illumination on the vertical frame-board, a card being previously placed in the exact plane to be occupied by the prepared plate. The image is now focussed on the card. Supposing the operation of exciting the plate to be done in the same room, sufficient light for the purpose is admitted through a small pane of yellow orange non-actinic glass let into the top part of the shutter. When ready the card is removed and placed against the open end of the microscope tube, so as to cut off all light through it, the plate is drained and placed on the vertical frame, the card quickly lifted and replaced against the end of the tube in periods varying according to the time of exposure necessary from part of a second to half a minute. The time required will vary according to the quality of the light, the sensibility to it of the collodion or other material used, and the facility with which the actinic rays pass through the object.

Mr. Wenham enumerates several advantages combined in this method. The length of base-board is limited only by the dimensions of the room. The ease with which any object can be included in a definite space. Facility in focussing—a means of so placing the card or sensitized plate at any angle to the axis of the microscope that the surface may be made parallel to objects lying a little out of one plane, and by having a series of paper stops at hand, parts situated in planes, slightly removed from each other, can be focussed and impressed alternately. Then while the first part is being impressed, the other part is stopped off, this is then stopped off, the other part focussed and its image allowed to fall in its turn on the unaffected portion of the prepared plate. Again the thicker and thinner parts of the same object may be exposed for different periods of time, by which a uniform intensity may be obtained in spite of the different transparency of different parts.

For the low powers the plane mirror, but for the  $\frac{1}{2}$ -inch objective and higher powers some form of condenser is used, as a bull's-eye lens, about 3 inches diameter. But for the finer forms of objects, as diatoms, the bull's-eye lens is to be combined with an achromatic condenser of the form proposed by him in April, 1861,

Fig. 216.



§ 251.

Fig. 217.

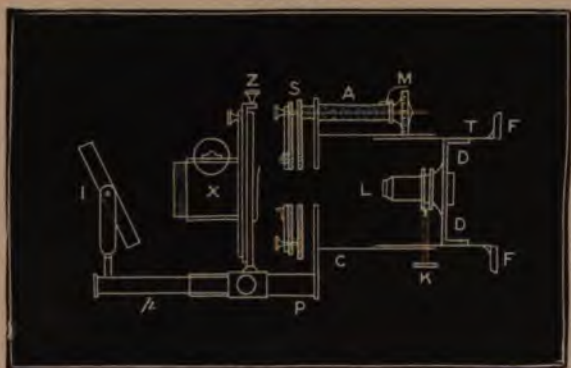


Fig. 218.



- Fig. 216. Photographic microscope camera, used by Mr. Delves and arranged by Mr. Highley.  
 Fig. 217. Stage, mirror, condenser, with adjustment to fit on the end of the above camera, as recently improved by Mr. Highley.  
 Fig. 218. Another arrangement with solar condenser. Mr. Highley.



for his binocular microscope. This consists of a set of three plano-convex lenses varying in diameter from about  $1\frac{1}{2}$  inch to  $\frac{1}{2}$  an inch, placed near to each other with their flat surfaces towards the object. These combined possess a very large angle of aperture. The small lens being made separable from the others, a large field of illumination could be obtained for the lower powers.

**253. Camera applied to the ordinary Microscope.**—We now enter on the plans for employing the microscope and camera united. Mr. Shadbolt recommends the draw tube, if any, to be removed, and its place supplied with a lining of black velvet. The microscope is now arranged and fixed horizontally on a board or table, and the body made to correspond to the centre of the aperture left on the removal of the lenses from the brass setting of an ordinary camera. The intervening space being closed in such a way as to exclude all entrance of extraneous light. The draw chamber of the camera is employed to vary the distance of the image from its object, but is usually deficient in length, hence some plan for elongating this chamber is needed. Many complain that when using the microscope in this way, some uncertainty in the centering and liability to derangement when exchanging the focussing screen for the prepared plate, are experienced. Gerlach adapts the camera to the top of the tube, the microscope being placed upright. (Plate XLVIII, Fig. 228).

**254. Dr. Maddox's Camera.**—The instrument proposed by Dr. Maddox, and used by him with considerable facility and great success, consists of a microscope having a compass-joint at the lower end of the stem furnished with coarse screws, &c. The stage slides along the stem and can be clamped to it by a binding screw acting against a guide that runs along its length. This stage is provided with small rectangular movements attached to the part holding the object slide, and to its opposite side is fixed a stout tube to hold an achromatic or some form of condenser. The main part of the stem is hollow and receives a strong tube furnished nearly in its entire length with a slot that works on an internal guide fixed inside the stem. This tube carries at its near end an arm, at right angles to which a tube about five inches long is screwed on the near side, and on the opposite side is fitted an adapter to receive the screw-end of the objective. An approximate focus is effected by sliding the stage along the stem, and the fine motion by a graduated milled-headed screw-pin.



This pin passes through the tube to which the arm is fastened and engages in a thread cut in the solid end of the stem. A spiral wire coiled in the inner tube reacts on the arm when the milled-headed screw is withdrawn. The whole of these arrangements are fixed firmly by the screw and nut at the jointed ends of the stem to a rectangular cross piece of 3-16ths iron bar about two inches wide, the screw passing through a hole near its centre. This cross piece is turned down at right-angles on each side so as to bring the centre of the short microscope tube in the centre of the camera, then again turned at right angles and firmly screwed to a stout base-board of deal  $1\frac{1}{4}$  inches thick, 12 inches wide, and 48 inches long, and clamped at each end to prevent warping. This is supported over a wide moveable triangle (Plate XLVII, Fig. 220) having stout double-hinged triangle legs of a height convenient for the operator (3 to 4 feet). About 12 inches from the end of the base-board where the microscope is fixed, is hinged a stout square frame with a sliding door having a central aperture to allow the end of the microscope tube to work through, the inside of the aperture is lined with leather, and a thick velvet collar is made to slide along the tube and abut against the aperture in the door, so as when in use to effectually cut off the entrance of any extraneous light. The frame with door is turned on its hinges, until it stands exactly at right angles with the axis of the microscope, and is kept firmly fixed in this position by two stout brass struts with clamping screws, that rise from the base-board on each side of the frame at an oblique angle  $60^\circ$ . At the opposite end of the stout plank is placed an ordinary camera with a moveable door-front having a *large* central aperture. One end of an expanding bellows body is fastened to it, the other end being attached to the door that slides into the vertical frame. This bellows part is made of two thicknesses of black twilled calico, having pasted between them a corresponding sized sheet of stout brown paper, and folded into one-inch plaits when damp, then turned over square to the size corresponding to the sliding doors, the corners bent down like the bellows of a common accordion, and the overlapping edges which are turned so as to face the base-board are double sewn together throughout their length; or for this may be substituted a body of black calico, of treble thickness, attached at each end to the doors, and kept apart laterally by elastic bands sewn along its four edges, lengthwise. The camera is made to slide along the supporting board between wooden guides screwed to its

upper surface near the sides, extending from the near end to the vertical frame. These have small holes at corresponding equal distances of half-an-inch, and projecting from each side of the body of the camera is a pierced horizontal ledge of brass plate, about 5-8ths wide, that travels over the upper surface of the guides on the to and fro movement of the camera, a moveable pin on each side fixing it in the place desired. These apertures are numbered according to the inches 1, 2, 3, &c., from the frame, and thus are of service to note the distance at which the sensitized plate is placed from it or from the stage. Memoranda being kept, the same ranges can be easily repeated. The draw chamber of the camera has its own focussing screw which is of use occasionally, but it is not necessary.

Two diaphragms of blackened stout card are placed within the chamber of this elongated camera, one near to the vertical frame or at the junction of the bellows part with it in front, and the other is placed in a grooved frame, that slides in a wide cut made in the inner surface of the underside of the draw part of the camera. This frame holder takes diaphragms with various sized apertures, according to the dimensions of the image of the object or the glass plates employed. Sliding this forward or backward in the camera alters the relative size of the field according as the camera is used expanded or closed. The camera is either dead blackened or lined with black cotton velvet, and the tube of the microscope inside is well covered by optician's charcoal black, or lined with black velvet, which is better.

The mirror or prism is set on a separate arm fixed to the base-board, in a line with the stem of the microscope, so that the axis shall correspond with the axis of the objective. The apparatus can be put together very quickly, or kept ready for use, and is of a size that permits of its being moved about easily, without being too cumbersome for one person; and it possesses considerable firmness.

The microscope portion can be supplied by any form of microscope that will take the horizontal position, and permit the eyepiece end of the body to work through the central aperture in the front of the bellows-chamber, provided means are taken to effect rigidity, and completely shut out the outside light around the aperture when working the rack for the coarse adjustment. But preference has been given by Dr. Maddox to a tube shorter than the usual body of the ordinary microscope, which sometimes

narrows the field too much when the camera is nearly closed on the vertical frame. The tube consists of two parts, one an inch in diameter, fixed to the arm, the other  $1\frac{1}{4}$  inches in diameter, that slides through the aperture in the door. On the open end of the latter fits a dead blackened brass cap, from the inside, with a slight internal projecting ledge, which acts as a diaphragm with a large opening.

The description will be more easily understood by a reference to Plate XLVII, which represents the instrument partly in section. The camera, when drawn out to its full range, has this difficulty, that it obliges the operator to withdraw the head from the focussing screen at the time of making any alteration in the fine motion. A lever arrangement has been used to obviate this, but if employed with the high powers demands extreme care if there be any slip to the screw. Mr. Legg employed a lever crank and arm over the top of the camera, working on the milled head of the coarse rack and pinion motion. Prof. Rood, of Troy, N.Y., also made use of a rod and lever beneath the camera, acting on the rack work, and a hinged mirror placed this side of the ground glass to receive the image transmitted to it while arranging the object on the stage plate and attending to the illumination.

Dr. Maddox has recently introduced the following plan for focussing in the case of his arrangement. A long brass rod, with a reel fixed on the front end, is passed through a hole drilled through the centre of the cross-piece to which the front legs are attached, this end of the rod being conical, to work easily in a hole made in a piece of brass plate screwed to the middle of the under surface of the base-board; the other end of the rod, with a fixed handle, having a groove cut in it, hangs in a collar screwed to the base-board beneath, in the median line. This permits of easy rotation between the fixed points. A piece of vulcanised india-rubber tube is drawn tightly over the reel. In the middle line of the base-board, just below the part where the milled screw traverses backwards and forwards in making the focus, a hole with slanting sides is cut through. A light brass double beam arm hangs on the milled head, and is clamped, when required, by a small clamping screw at the centre, to the centre of the milled head. A band is passed round the reel through the aperture cut in the board, and each end fixed to an arm of the beam. When the ordinary focus is made with the camera closed



Fig. 219.

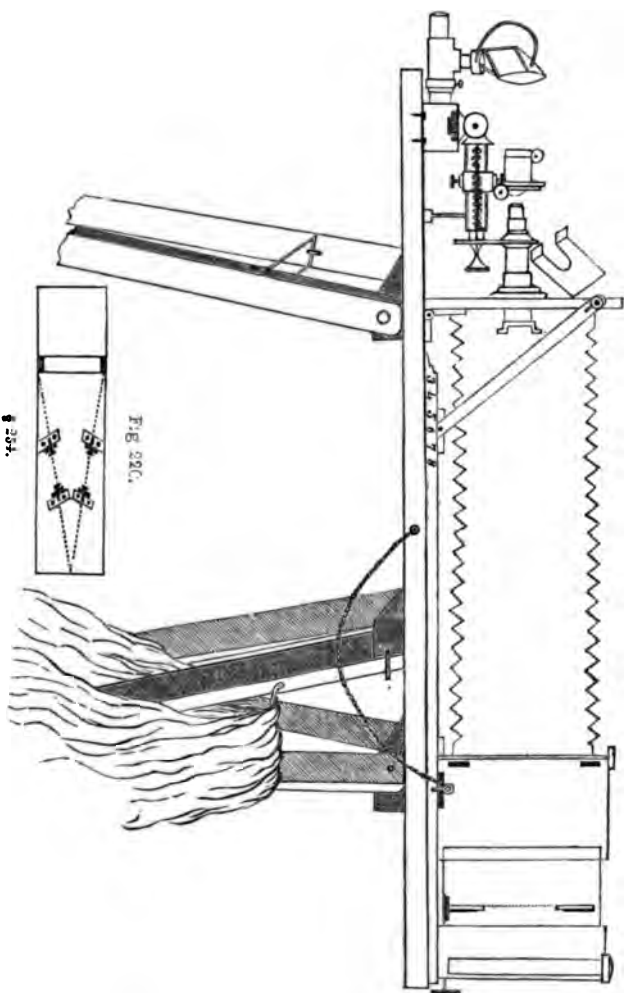


Fig. 220.

Fig. 221.

Fig. 219. Photographic camera as arranged by Dr. Muller, described in page 138.  
Fig. 220. Under-surface of base board to show the position of the legs which support the camera.





up the clamping screw is turned to release the beam, and the focusing-screw now rotates easily; when the camera is drawn to the distance required, the clamping screw is made to fix the beam. The focussing eye-piece is held by one hand, while the other turns the rod beneath. If in the first focus the object be well focussed into, on withdrawing the camera a very slight alteration will be needed by the use of the rod. This arrangement is not represented in the plate.

Instead of the beam, if a grooved pulley-wheel is fixed on the upper surface of the base-board, the milled head having a groove cut in it for the band, the necessary motion can likewise be easily obtained.

The chief requirements in any form of camera, independent of the objective or mode of illumination, are general facility of management, compactness within a moderate range of extension, correct centering, *absence from all vibrations*, and the total exclusion of all light except that which enters by the object-glass.

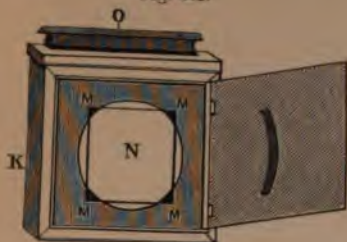
**255. Arrangement of Drs. Abercrombie and Wilson.**—I am enabled, through the kind permission of Drs. Abercrombie and Wilson, of Cheltenham, to mention the plan that has been so successfully adopted by them, and I am glad to find that Dr. Wilson is about to give, in one of the current journals, a detailed account of the system, which on account of its simplicity, the employment of artificial illumination, and the beautiful photographs which have been obtained by it, possesses special merit. These gentlemen use a stout plank, 6 or 7 feet long, having parallel strips of wood along its entire length. At one end, between these, slides a piece of wood, to which the microscope is clamped, and on which the light is placed, so that light and microscope may be moved together. The camera is raised the necessary height on a block, and slides at the other end of the board. Thus both can easily, still being centered, be made to approach or recede from each other singly or together, but it is considered best, as a rule, to have the camera fixed, while the microscope travels along the board. Two thin strips of blackened wood, diverging from each other and connected by parallel cross pieces, are fixed at the narrow end to the tube of the microscope with cotton wool in such a manner that the extraneous light is totally excluded. The diverging end passes to the top of the camera. Over this frame is thrown a large piece of black cotton velvet, pile-side

inwards. They have used an oil-lamp, and think this sufficient for images enlarged 150 diameters, but for higher powers, as 400 diameters, they prefer the oxy-calcium light.

The direct rays from the source of light are condensed by one of the Rev. Mr. Reade's kettle-drum or hemispherical condensers, either directly or after being first collected by an ordinary bull's-eye lens of some 3-in. focus. The time of exposure for wet collodion plates varies, increasing according to the colour of the object, and its enlargement;—50 diameters and a tolerably light object may need ten minutes with the oil-lamp. By placing a small vessel of warm water in the camera, to keep the collodion plate moist by its vapour, they have exposed plates for forty minutes with success. Some of the prints from these gentlemen's negatives are remarkably good; they possess a peculiar delicacy in the half-tones and shadows, with much roundness of the objects, but the definition, as might be expected, does not quite equal, in some of the finest markings, prints obtained from sunlit negatives. However, all of the general characteristic appearances of the objects are exceedingly perfect. Great simplicity in the apparatus, and the immense advantage of useful illumination in all weathers, are most favourable recommendations.

**256. Of the Illumination: Sunlight.**—Both sunlight and artificial light have been used. Dr. Maddox, with the majority of observers, gives the preference to sunlight in all cases, and nearly always uses some form of condenser. He usually dispenses with the mirror, and substitutes one of Abraham's achromatic condensing prisms, placed at such a distance from the object (if used alone) that its rays would cross just before reaching the object. Otherwise the intense heating power at the vertex of the cone of rays would cause considerable danger to the object, and might uncement the lenses of the objective of the higher powers, especially when the object is only enclosed between two pieces of the thinnest covering glass, and the focus very close. The prism he seldom employs alone, but places in the tube at the back of the stage a condenser. A small Coddington lens, about  $15^{\circ}$  angular aperture, served him in the earlier part of his experiments. This was made to slide in a tube nearer or farther from the object. Latterly he has used Sollitt's achromatic condenser, as furnishing a larger field and more free from spherical aber-

Fig. 221.



§ 267.

Fig. 226.



§ 267.

Fig. 222.

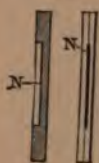


Fig. 227.



§ 256.

Fig. 223.



§ 273.

Fig. 224.

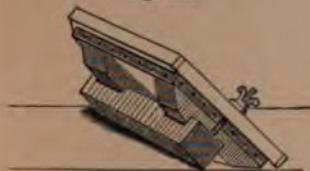


Fig. 225.



Fig. 228.



§ 253.

Fig. 221. Photograph plate holder, after Mr. Highley.

Fig. 222. Sectional views of glass and wood plate holders.

Fig. 223, 224, and 225. Three views of a pressure frame used in photographic printing.

Fig. 226. Arrangement of the lenses used for condensing the light of a lamp, as arranged by Mr. Shadbolt.

Fig. 227. Arrangement for obtaining parallel rays, as recommended by Gerlach.

Fig. 228. Mode of adapting the camera to the microscope adopted by Gerlach.





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ration. This condenser, as described by the originator, consists of two achromatic lenses with their plane surfaces turned towards the object, and of 2 and 4 inches in their respective foci, placed at the distance of one and three quarters of an inch apart with a diaphragm between them. The four-inch focus lens has a diameter of  $1\frac{1}{2}$  inch, the two-inch focus lens a diameter of  $\frac{3}{4}$  of an inch. Here then we have a large body of light, and a field beautifully illuminated when used either with the plane mirror or the prism. A series of diaphragms slip into the cap covering the small lens, which is turned towards the objects. Sometimes Dr. Maddox employs an achromatic doublet of about  $22^\circ$  aperture, or an achromatic condenser of larger angular aperture. Although theoretically the angular aperture of the higher objectives is narrowed by these moderate apertures, practically the intensity of the illumination appears to compensate in a remarkable manner, as is shown by the perfect delineation of some of the figures in the frontispiece. The common plan is to use as a condenser the objective next below the one used to render the photographic image; but if any form of solar condenser be employed by which the rays become much concentrated, the greatest care will be required, to avoid injury to the lenses by the intense heat.

Dr. Maddox has lately used the condenser of three plano-convex lenses as recommended by Mr. Wenham, but employs with them moveable diaphragms; these are placed nearer to or further from the largest lens, the distance being regulated by trial.

Prof. Rood, of New York, for his higher powers made for his condenser a Wollaston doublet, having an angular aperture of  $44^\circ$ . He used one of Liebig's silvered mirrors in place of the ordinary amalgam mirror.

M. Neyt replaces the common solar reflectors by a large prism with parallactic motions; to condense the rays an achromatic condensing lens of  $2\frac{3}{4}$  inches diameter is used, and to concentrate them still more, 3 other converging lenses are placed in its focus in such a manner that they can be used together or separated to meet the power of the objective. He likewise has the objective corrected to make the chemical and visual foci agree. In order to render infusoria stationary while they are photographed, he uses a voltaic stage, so that he can make contact with the poles of a Daniel's battery or induction coil at the proper moment. The shock suddenly kills the little beings and enables him to

secure an image, when otherwise, from their rapid movements, it would be a mere accident if the animalcule being in the field of view, or in the desired attitude.

The Rev. Mr. Reade has proposed a very ingenious method of using his hemispherical condenser with a solar condenser. The rays furnishing light and those giving heat having different degrees of refrangibility, we have here the cone of light-giving rays formed within the cone of the heat-giving rays, the principal focus of the latter being at a greater distance from the lens than the former. When these rays are permitted to cross the axis, their respective situations are reversed. On arranging the hemispherical lens, so that it shall be separated from the principal focus of heat by the sum of its own focal length, the principal focus for light will be found at a greater distance than its own focal length; hence the heat-giving rays will be rendered parallel, and the light-giving rays will be made to converge to a second focus furnishing light of much intensity separated from the heating rays, thus supplying means for using an achromatic object-glass for the solar microscope without endangering its injury.

Professor Gerlach uses a plano-convex lens with a concave mirror. These are placed at such distances apart that the two foci meet when the convex surface of the plano-convex lens is turned towards the mirror. By this arrangement parallel rays are sent through the object. (*See Plate XLVIII, Fig. 227.*)

In the ordinary Wollaston doublet the chromatic aberration is not corrected, but this does not cause any serious difficulty, as by varying its distance, the blue or chemical end of the converging cone of rays can be used to furnish a field of bluish light. Some considerable care is needed in the adjustment of the condenser, whichever kind be employed, so as to equalize the illumination and avoid sun spots when the mirror is used. Mr. Traer got rid of these by making the distance between the object and concave mirror rather more than its focus. The chief object is to have the full amount of light that will furnish a distinct image on the ground glass. Some make their focal arrangements in the objective, illuminating the object with daylight or a less intense illumination than is to be used in taking the photograph. Dr. Maddox found that in doing this he seldom secured the best focus, therefore he prefers to focus in sunlight condensed upon the object; now the eye will often

catch some fine line or mark distinctly on the ground-glass screen, even without the aid even of an examining eye-piece, that could not be seen by daylight illumination, nevertheless it is generally desirable to use this little instrument.

**257. Artificial Light.**—Mr. Shadbolt many years ago obtained some beautiful photographs by lamp light. A small camphine or paraffin lamp was placed so that the flame was in the axis of the microscope. A plano-convex lens of about  $1\frac{1}{2}$  inches diameter with its flat side to the lamp, and a second smaller one of about 1 inch in diameter and 3 inches focus, were arranged so as to concentrate the rays of light without forming an image of the flame (Plate XLVIII, Fig. 226). The first is placed at such a distance from the lamp as to make the rays converge slightly, and the other at a point where it will include all these rays and (in using high powers) the achromatic condenser, so that the lens may fall well within the cone of rays. In employing low powers the object is made to come within the cone of converging rays. The distance of the lamp from the nearest lens to it, is best determined by the quality of the illuminated field, which should be equally bright, nor should the light enter the objective at a greater angle than its own angular aperture. To examine the image thrown on the ground-glass of the camera, Mr. Shadbolt used a Ramsden's positive eye-piece.

Mr. Legg, in 1859, made use of artificial light from a camphine lamp, concentrating the diverging rays by a two-inch bull's-eye lens placed near to the source of light, and a second bull's-eye lens about three inches in diameter at a distance of an inch from the first, by which, with the 2-3rds and 4-10ths object-glasses, he could obtain images at 3 feet, in periods varying from 3 to 10 minutes.

Mr. Parry, in making use of artificial light, placed a plano-convex of  $1\frac{1}{2}$  inch focus with its plane side towards the object about one inch from it, and four or five inches from an argand gas burner. The light from an argand paraffin lamp is preferable to gas. To increase the flatness of the field, he fixes behind the posterior lens of the 1-inch combination, an achromatic stereoscope camera lens with its flat surface towards the objective. The advantage of the brilliant light produced by the combustion of magnesium wire has been referred to in page 152.



**258. Of Focussing.**—Much care is required in focussing. A plan adopted by some is to use a simple lens set as a watch-maker's loupe in a card or wooden tube of such a length, that when placed at the near surface of the ground-glass screen, the focus of the lens exactly corresponds to the opposite or ground side. Others employ an ordinary photographic focussing piece. The best is the positive eye-piece, for should the others not be truly set, there is danger of the focus catching the image either before or behind the screen.

**259. Of the Object-glasses.**—Each objective, as furnished by our best opticians, is generally sent out not as a *photographic* object-glass, but set as a microscope objective, and so skilfully have errors which arise from the thickness of the thin glass cover and non-achromaticity of the eye-piece been compensated for, that the illuminated field is without sensible colour, and the edges of objects destitute of chromatic fringes. To accomplish this, the objective is left what is termed "over corrected."

When the photographer employs these objectives, more especially the low and middle powers, he finds that either his prepared sensitized plate must be moved further away from the plane at which the best visual focus was found, or else he must withdraw his objective a slight distance from the object, and bring the chemical focus to its compensating point for the amount of "over correction" that has been given to it by the maker. This is not a fixed sum, and may vary in different object-glasses furnished by the same optician, when of equal magnifying power, or even ground on the same tools. In the construction of some of the lower powers a plan has been adopted which, at the same time that it does not detract from their optical perfection, places the chemical variance at its lowest mean. In the higher powers, as from  $\frac{1}{4}$ th upwards, the difference between the visual and chemical foci is so small that it is seldom regarded, except in the most delicate work; but here the disturbance occasioned by the cover of thin glass placed over the object, requires the adjustment between the two posterior combined sets of lenses, and the anterior pair or triple lens to be made with the greatest nicety, and even then if the best definition is to be obtained in the photographs, a very slight alteration of the screw collar will be required. It is not possible to determine beforehand the amount of alteration in focus needed, and a series of trials will

be necessary to establish what adjustment is requisite. The best plan is to select an object that has a slight thickness, with parts at a distance from one another, lying in three or four different planes. Set the objective to the best focus in the microscope, then place it in the camera; focus sharply for the part of the object nearest, and in the negative which is taken observe if this part corresponds in definition, or if not, which plane of the object appears the sharpest. Let us suppose the furthest plane; then observe, by re-focussing, how many divisions of the milled-headed screw have been turned through to bring this part into as perfect a focus as was originally the nearest plane. This will give the variation for that objective under similar circumstances, and should be noted. If employed with the deeper eye-piece, to increase the magnifying power, with the loss of some definition, from the under correction of the eye-piece, a different adjustment will be required. Mr. Shadbolt undertook a series of experiments for his objectives, of Messrs. Smith, Beck, and Beck's make, when he employed artificial light, and which he gives as follows:—

The  $1\frac{1}{2}$ -inch object-glass to be withdrawn  $\frac{1}{30}$ th of an in.

The  $\frac{3}{4}$ rd.                   "                   "                    $\frac{2}{100}$                    "

The  $\frac{1}{10}$ th.                   "                   "                    $\frac{1}{1000}$                    "

These can only be regarded as guiding marks for others. To obviate this great inconvenience, Mr. Wenham, to whom we owe much for the perfection of the binocular microscope, with his usual ingenuity, recommended a biconvex lens of low power to be carefully turned down to the proper size, and centered in a setting that can be screwed into the place where the posterior diaphragm or stop is usually placed; thus to lessen the over correction and to bring the chemical back to the visual focus. He gives the following focal lengths of these correcting lenses for Messrs. Smith, Beck and Beck's  $1\frac{1}{2}$ -inch, a lens of 8 inches, focus for the  $\frac{3}{4}$ rd; one of 5 inches focus, which is also applicable to the  $\frac{1}{10}$ th.

Mr. Hislop advises that a dozen of these, of different foci, should be at hand, and the one that is found to answer best in practice selected. Dr. Maddox has one of Messrs. Smith, Beck and Beck's  $\frac{3}{4}$ , beautifully corrected by them in this manner, and it gives surprising sharpness.

Mr. Shadbolt prefers to find the necessary alteration for the foci of different objectives. It seems almost a matter of regret that opticians have not offered a special correcting eye-piece to

hole in the small strips of spring brass, then through the holes in the ledges, the pins being now turned up at right angles to prevent being carried out of the holes by their springs. An ordinary glass slide with the object set up was placed between the springs (and rested by its under surface near the edges on the upper or horizontal surfaces of the two small blocks), being clipped by them sufficiently tight to prevent falling out, when the slide was placed vertically on edge. On depressing either end of the slide-holder, the object could be made to assume an obliquity to the objective, equivalent to the angle found between the surface of the little triangular blocks and the edge of the depressed slide when resting on the plane of the brass holder. This method answered very well for opaque objects illuminated by the Lieberkühm. The slide holding the object being first centered and focussed, then depressed and re-focussed if necessary, to furnish the first picture, then similarly treated on the opposite side of the centre to furnish the second. The resultant images giving a stereo-picture; when the left depressed view is taken on the right-hand side of the plate, and *vice versa*, the images need not be reversed after printing. He also used for transparent objects Mr. Wenham's and Mr. Smith's plan for stopping off alternately in front the right and left halves of the objective by a small cap with a semicircular aperture, equal generally to half the area of the front lens, while with the highest powers, he only makes a slight alteration in the position of the object and incident light for the second picture. With the parabollic illuminator he did not succeed equally well. M. Nachet, jun., used, if we remember correctly, his polished cone of glass with a central stop on its flat surface (for obtaining oblique light in parallel rays) when photographing opaque objects, as the Foraminifera.

#### CHEMICAL SOLUTIONS REQUIRED.

The different solutions used in photography must be perfectly pure; this is of the first importance, and observers are recommended to purchase their chemicals at houses of known celebrity, rather than attempt the manufacture.

**261. Collodion.**—Supposing the collodion process to be determined on, the pyroxyline should be of the kind produced from



hot acids, carrying just such an amount of water as will furnish to it when dissolved in its solvents, æther and alcohol, a fluid flowing freely, possessing considerable adhesive power to the glass, and free from fine net-like markings when dry. The manufacture of the gun cotton that will furnish these qualities requires great experience. The collodion should afford, when taken from the nitrate bath, not a very thick creamy layer, but one slightly thinner than that commonly employed for portrait purposes. If it be preferred to make the collodion, we subjoin the formula for cotton that will yield the above mentioned film. Into a perfectly clean dry close stoppered bottle, put—

Iodide of ammonium in crystals.\*

Iodide of cadmium, of each, 8 grains.

Bromide of cadmium, 4 grains.

Pour on these 13 drachms of absolute alcohol or redrawn alcohol, of sp. gr. .805, shake the bottle well; when dissolved, add—

Pure æther, sp. gr. .725, 12 drachms.

Weigh out 22 to 28 grains of dry pyroxyline, add it by little open tufts to the mixed fluid, shaking occasionally, then wash down the neck and sides of the bottle with 8 drachms of pure æther. Gently agitate so as not to soil the neck of the bottle, and set aside in a dark cool cupboard for three or four days, or longer; then carefully pour off, without any shaking, the half into a clean dry close stoppered bottle for use, or better, into one of the 4 oz. capped pouring bottles, called "cometless." The formula given has been for only 4 oz. of collodion. The absolute alcohol can sometimes be a little increased.

**262. Nitrate Bath.**—The nitrate bath may be prepared as follows:—

Freshly distilled water, 4 ounces.

Recrystallized nitrate of silver, 600 grains.

Dissolve; test for acidity by blue litmus paper; if acid, neutralize by a few drops of a very weak solution of carbonate of soda; dissolve in a drachm or two of water—

Iodide of potassium, 1 grain.

\* If the crystals of iodide of ammonium be at all damp, press them before weighing in folds of clean blotting paper.



Then drop into it a few drops of the strong solution of nitrate of silver until it produces no further turbidity. Wash the precipitated yellow iodide of silver, pour off the washings, and add the iodide to the strong silver solution; stir, make up the quantity of fluid to 20 oz. by distilled water, and filter if it appear at all turbid; but if not, it is preferable to allow it to settle, then carefully pour off close, and filter the remaining portion into a small bottle. This can be used in the after intensifying process, or if filtered through a washed filter, added to the stock for the nitrate bath.

The strong solution of silver is oftener rather alkaline than acid to test paper, if this be the case, add a few drops of a solution containing 1 drop of glacial acetic acid to 1 drachm of distilled water, until the test paper remains slightly reddened, or the same proportions of nitric acid in water may be used; the latter often works remarkably well with the bromo-iodized collodion, not giving intense, but remarkably sharp clean negatives, permitting of a rather longer exposure to the strong sunlight without staining, and considerable intensifying qualities without blocking out the finest lines. To keep up the strength of this nitrate bath, add occasionally a plain solution of recrystallized nitrate of silver in distilled water, in the proportions of 40 grains to the ounce.

It is desirable to keep this nitrate bath perfectly free from dirt or such bodies as are likely to injure it. As there is considerable difficulty in obtaining the gutta-percha baths without impurities, and the porcelain ones are sometimes too porous, a vertical glass bath with cover is much to be preferred. It is often useful, after a full day's work, to pour the nitrate bath into a clean bottle, allow it to settle in this, and then carefully decant the clear portion into the bath, after it has been washed out, and filter the remainder through a washed paper filter, making up the strength, if necessary, by the 40-grain solution. In this way there is less likelihood of spots, pinholes or deposit on the transparent shadows.

This bath in winter can be made stronger, and in summer may be allowed to fall a little lower.

**263. Of the Developing Solutions.**—Preference is given to the formula containing the protosulphate of iron, or the double salt of sulphate of ammonia and iron.

Crystallised protosulphate of iron crushed, 200 grains.  
 Glacial acetic acid,  $3\frac{1}{2}$  to 5 drachms, or,  
 Beaufoy's acetic acid of 30° per cent., 10 to 15 drachms.

The amount of 10 oz. is to be made up with pure water, then 6 or 8 grains of acetate of soda are to be added, and the fluid filtered. More iron should be added to this developing solution in the winter months. It is best when a few days old. At the time of using, to make it flow freely on the surface of the colodionized plate, add of ordinary alcohol from 20 to 30 minims to each ounce of developing solution, according to the condition of the bath.

The intensifying solution, useful for deepening more fully many of the details brought out by the iron developer, consists of:—

No. 1. Iodine, 3 grains.  
 Iodide of potassium, 6 grains.  
 Water, 3 ounces. Mixed.

No. 2. Pyrogallic developing solution, as made with acetic acid.  
 Pyrogallic acid,  $1\frac{1}{2}$  to 2 grains.  
 Glacial acetic acid, 20 minims, or  
 Beaufoy's acid, 1 drachm.  
 Distilled water, 1 ounce.

This is best when freshly made, or not more than a few days old.

No. 3. Pure nitrate of silver, 30 grains.  
 Distilled water, 1 ounce.

No. 4. Pure nitrate of silver, 20 grains.  
 Citric acid crystallized, 30 grains.  
 Dissolved in distilled water, 2 ounces.

**264. The Fixing Solutions** may be made as follows:—

Hyposulphite of soda 4 oz., dissolved in 4 oz. of water. Using it repeatedly until saturated with the dissolved out iodide and bromide of silver; but we prefer a fixing solution made by dissolving about—

8 grains of cyanide of potassium in one ounce of water.

It should be marked **POISON**. As this substance varies in its strength, the solution should be made so as to clear the plate

in a gradual manner in from one to one and half minutes, but not so strong as to destroy the half tones. The same solution can be used repeatedly, or until rendered, by using, too weak. It should not be kept exposed to the air.

#### PRACTICAL MANIPULATION.

This is naturally divisible into three distinct stages : 1, obtaining the image on the sensitized plate ; 2, rendering it visible : and 3, obtaining a print upon paper.

**265. Cleaning the Glass Plates.**—The glass plates, whether of "patent plate," which is the best, or of "polished flatted crown," are first to have the sharp edges removed by a grooved roughening stone sold for the purpose: this is best done under a gentle stream of water from a tap, that the particles of grit or dust may be carried away: the plate is then dropped into a clean pan containing a hot solution of washing soda in rain or soft water. After lying in this for a little time, they are singly washed over back and front with a pledget of tow and a saturated solution of washing soda, then dropped into clean hot soft water. When all the plates have been treated in this way, they are taken out to drain, the water thrown away and fresh hot water poured into the vessel. The plates are singly dipped under the surface of the clean water, then wiped with chemically clean linen cloths, such as old napkins, one covering the left hand on which the plate rests, the other being used to dry and polish the plate. These cloths are *not to be washed with soap and water*, but to be well washed out in *hot soft water*, containing a little soda, then well rinsed in fresh water and dried.

It is well to keep a stock of plates thus partially prepared. To further clean them, examine the plate along the edge, and if any very slight curvature exist, let this be taken as the surface on which the collodion is poured. Select three chemically clean dry cloths, fold one into double thickness, and on it hold the plate in the left hand, face down ; with one of the other cloths polish well the back, breathing on it from time to time ; then turn it face uppermost, have a little old collodion which may be slightly weakened with alcohol, place a pledget of clean cotton wool in a small cleft stick or whalebone, dip it into the old



collodion, pass it quickly and well over every part of this surface of the plate. With the same cloth that polished the back, rub this off briskly, then with the other clean cloth finish off the polishing, so that when breathed on the surface may present a uniform dull appearance without any streaks; set it face down on a clean sheet of foolscap paper, or in a grooved well-closed plate box, the finished faces all looking one way. Thus prepare the number of plates required for immediate use. If to be kept a few hours, wrap them up in another fold of paper, place them in a dry drawer, always well noting which is the perfectly cleaned surface. If of a larger size than 6 inches square, it will be more convenient to clean them on a proper polishing board. Cleanliness in this, as in the succeeding stages, is absolutely requisite. If no old collodion be at hand, a polishing liquid may be made by—

Howard's precipitated magnesia, 20 grains.

Strong liquor of ammonia,  $\frac{1}{2}$  drachm.

Alcohol, 2 ounces.

This, however, requires to be most carefully removed, in the cleaning, from the edges of the plates, or they would soon render the bath alkaline.

**266. Arranging the Camera.**—Supposing the form of apparatus recommended by Dr. Maddox be selected, we proceed as follows:—A room is to be chosen which has a window with a south-west aspect, or at least one where the sun's rays enter the greater part of the day. The end of the apparatus is placed against the opened window in such a manner that the face of the prism is directed at right angles to the incident rays; the legs of the triangle are set apart so that the whole stands firmly on the floor. The object being fixed on, it is first carefully examined under the compound microscope, and if of any depth, the part in strict focus when the best general character of the object is attained, is well noted. The objective likewise being determined on, it is to be screwed into the adapter of the arm of the microscope. The achromatic condenser is to be placed in the fitting on the under surface of the stage-plate. The blackened card diaphragm, according to the size of the field desired, is to be fixed in the diaphragm frame that works to and fro in the cut in the back part of the camera chamber, and the prism so turned that the sunlight be thrown on the ground glass-screen. Then let the stage be brought up to the position it will occupy for the objec-



tive to be in focus, when the object slide is in situ. The value of the prism is now apparent, for standing with the face towards its convex surface, and turning it on its own parallactic motion, an intense image of the sun is soon found, as it were, on that surface: the prism is then to be so arranged that the reflected images from the lens or lenses of the achromatic condenser and of the objective, fall centrally on this sun's image. If the field on the ground glass now appears equally bright in all directions, the achromatic condenser is slightly altered, to see whether any increase of illumination accompany the change, if not, it is returned to its previous position. Should the images not all fall into the image of the sun, seen on the surface of the prism, some alteration must be made in the part which seems most at fault; but when they all fall into it, and the distance of the prism is such that its converging rays just cross before reaching the object, the probabilities are that the centering is correct. If the prism will not carry a cone of light sufficiently large and bright for the lowest powers, as 3 inches, then set it aside and try the plane mirror. The object, if in the ordinary  $3 \times 1$  inch slide, is now placed on the stage, the camera bellows-body shut up; the whole apparatus is covered with a large focussing cloth of black cotton velvet, except the parts to be exposed to the light, the right hand is applied to the slide, and the eyes directed to the ground-glass screen under the focussing cloth; the object is now placed nearly as possible in the centre of the field, and the approximate adjustment made. When once the distance between the objective and the object has been noted, with the camera nearly shut up, the stage can be made to slide upon the support and clamped there, and the fine adjustment left to perfect the focus. If by any change in the stage a diminution in the intensity of the illumination should occur, then alter the rack-work of the condenser and the prism until the best effect is produced, for the proper position of the condenser is a very important one, and often more trouble to arrange than the focus of the object-glass. The object being well centered, the field perfectly bright and uniform, see that the velvet collar around the microscope tube *abuts closely* against the aperture in the door of the vertical frame; now withdraw the camera along the base-board from the near end, and closely watch the enlargement; when this is determined on, fix the camera by the wire pins to the nearest hole in the two wooden guides. Supposing this to be at an easy working distance,—watch the image on the ground glass

through the focussing eye-piece; turn the graduated milled-headed screw of the fine motion until the same point as was previously noted is brought into a sharp focus. Should the over correction of the lens not have been carefully corrected by a back lens, as previously advised, proceed to make the necessary allowance, which experience has determined, by turning back the screw of the fine motion, the number of divisions or parts required as marked on the milled head. If not known, commence the experiment as before stated (§ 259), and note the particulars. A card covered with black cloth or velvet, with its lower edge turned at right angles and deeply notched, is now rested on the stem of the microscope against the end of the achromatic condenser, facing the prism, and this latter protected by a thick fold of chamois leather from the sun's rays. Care must be taken that neither surface of the prism is soiled by vapour nor finger marks; nor must the concentrated sunlight be permitted to remain longer on the object than is actually required in focussing, or it may become uncemented, and if not injured, it may slip completely out of the field.

This apparatus, if used in the open air, could have the microscope end to move instead of the camera, but this method is very inconvenient, when used near an open window, from the difficulty at times to place the prism outside the plane of the window or in its best position.

If the higher powers be used, needing the screw adjustment for the correction of the error introduced by the thin glass cover, we find it best to make this as nearly as we can when examining the object in the microscope, then testing, with the collar set to that figure, the image on the ground-glass screen. If the image here seems moderately sharp, under the best focussing, a trial is made by shifting the collar a very little and watching the appearance of the image; sometimes a very trivial alteration will bring out fine markings much more distinctly; the focus, if this be the case, will also often require its readjustment; but before making this, it will perhaps, if only trifling, be as well to test a plate, when, should the negative be found defective in the parts most sharply focussed, try another, withdrawing the objective by the milled-headed screw. It is often in this way that the qualities of an objective are rendered evident; in fact, it often becomes very troublesome to ascertain these points for a variety of objects and covers. Assuming that the plane of the greyed glass screen, and that occupied by the sensitized plate, are *strictly* alike, if the

second image be out of focus, test again with the apparent necessary change learnt from a close examination of the negative, and the image on the screen. When once correctly found, note the division of the screw collar and the distance in inches at which the camera stands fixed by the pegs, and seen by the figures on the guides, as necessary for that objective used at the same distance with sunlight, and for objects covered by glass of that substance. If the distance be much altered in photographing that object, the chances are, that another readjustment will have to be made in the screw collar to attain the best negative.

Dr. Maddox remarks, that when the edges of objects under the higher powers present, on the grey glass screen, a faint tint of claret on the one side, and of apple green on the other, that great sharpness will often exist in the negative; the errors of the pairs of lenses balancing one another as regards the actinic focus. The roughness of the screen will not, in many cases, permit of the eye determining under sunlight the best focus for the minutes markings, but hitherto nothing has been found more generally serviceable than the *finely* ground surface.

Should the object be situated any distance from the thin covering, *i. e.*, have much of the mounting medium included within that space, although the objective may visually appear to work fairly through the depth, it is seldom that the negative of the image proves satisfactory. It is far better to remount the object, or select another. Indeed, for the finer work, it is advantageous that the objects should lie closely on the undersurface of the thin cover, and if, of the diatomaceous class, they should be dried on it before being placed on the drop of balsam warmed on the glass slide; this may likewise be thin, and certainly should not be thick. If there be any vibration from unsteadiness of the apparatus, or from wind, the operation of determining the best focus will prove very troublesome.

**267. Inserting the Plate.**—The suitable sized plate of properly cleaned glass being selected (and the materials required being set at hand in the dark room or portion of the chamber, as a large cupboard, darkened off for this purpose, and lighted by a small oil lamp with yellow glass shade), the plate is held by the sides between the fingers and thumb of the left hand, face down, the back wiped carefully over with a dry wide flat camel hair wash tool, to remove small particles of cotton or dust; then taken hold by a pneumatic holder in the centre, and the face dusted



over with the brush. Before taking the holder in the left hand see that the neck and lip of the collodion bottle are perfectly free from any portions likely to be carried on to the plate by the stream (the finger is commonly passed over these parts to clear them away), pour the collodion with a steady flow on to the plate, a little nearer to the left hand than its centre; while flowing, depress the lower and upper left corners, gradually, to bring the collodion fairly to their edges, at the same time that the pool is being increased by pouring, and then lower the plate to flow the fluid to the right further corner, and pour off at the lower one into the bottle, resting the sides of the angle on the lip, and rocking it keep the plate slightly inclined; drag off, as it were, the lower part against the neck of the bottle, close it, and hold the plate horizontally by the pneumatic holder for 10 seconds to  $\frac{1}{2}$  a minute, or even more, according to the setting quality of the collodion; if this be slow, it will be better to rest the holder on some flat place or shelf, that the warmth of the hand may not cause unequal evaporation—once seeing this well done is better than a lengthy description. Detach the plate from the holder and place it on the fluted glass dipper, to be plunged at one gradual stroke into the nitrate bath. Here it is allowed to remain for 1 minute, then raised and lowered several times so as to wash the surface well, and permitted to remain in the bath for one or two minutes longer, when the dipper with plate is to be steadily withdrawn; the plate removed, and rested by its lower edge on a pad of clean blotting paper, the dipper returned to the bath, and the back of the plate wiped with a pledget of clean rag, being gently steadied by the top corners between the thumb and fingers of the left hand, which must be dry and clean. Open the back of the plate frame and place with the right hand the plate into the frame, which should be dry and free of dust, face downwards, close the back, cover the frame with a large piece of black calico, and carry it lower edge down to the apparatus, rest it against the wall or table. Re-adjust the prism, remove the focussing screen, having glanced at the image on it, set the covered card against the achromatic condenser, pass the slide holder under the focussing cloth; into the position of the greyed screen, *lift carefully* the shutter of the frame, the hands being under the cloth:—let all remain for a moment or two that vibrations may cease, snatch away the card without shaking, and replace it quickly, allowing a period from half to 25 or 35 seconds for the



image to be impressed ; the time must be learnt by practice ; close the shutter gently, withdraw and replace the frame in the cloth, pass the focussing screen into its place, again snatch away the card and observe the image, then cover the prism and return with the slide holder to the dark cupboard, and proceed to develop the picture. The reason for the re-observation of the image is, to see if the object and its focussing have not been in any way deranged, so that if the development brings out a good image, it can be repeated without the necessity of returning to the camera before the second plate is ready.

**268. Developing the Image.**—Let us suppose the plate to be a small one, first see that the nitrate bath is *carefully placed out of the way of all splashes*, pour into a clean developing glass an ounce or more of the iron developing solution, add the necessary quantity of alcohol, and mix ; remove the plate from the holder, rest it face up on a levelled developing stand set in a large basin or pan, clip the left hand opposite corners between the finger and thumb, and commence the second step by flushing the surface with some of the iron solution ; tip the plate about that the liquid may quickly flow up to all the edges, then move it gently about on the top of the stand : the light from the protected lamp falling nicely on the surface, watch for the appearance of the image ; this, if all be correct, will increase steadily up to a certain point, when, if left longer, the plate will begin to grey all over ; just before this would take place, or according to experience, tip up the plate to throw off the developer, examine it momentarily before the lamp, and if the image appear nicely out in the detail, flush the plate well with water to remove all the iron. Now examine the plate more carefully by transmitted light from the lamp with yellow shade, and judge if it be worth continuing the other operations ; reflush again with water, pour off, and now pour on the fixing solutions—we give the preference to the cyanide of potassium—pour this along the thickened part of the collodion, let it pass over all the plate, and in a minute, or a little more, the plate will be cleaned of the unaltered bromo-iodide of silver. Wash well front and back with clean common water from a jug, or small tap, protected by a piece of flannel tied loosely over it, drain the plate for a moment, and pour on it along the edge sufficient of the intensifying solution of iodo-iodide of potassium, to well cover the surface ; allow this to remain on

the plate until the grey color of the image passes to a warmer tone (two or four minutes or more), pour off the fluid, carry the plate to the light, examine it quickly with a hand magnifier; if it now has the appearance of being well in focus, return to the closet, wash the plate well with common, then clean with fresh rain or distilled, water; let this stand on it whilst you pour into another *clean* developing glass about  $2\frac{1}{2}$  or 3 drachms of the pyrogallic solution; add to this from 6 to 10 drops of the 30-grain nitrate of silver solution, and 2 to 4 drops of the citro-silver solution, mix these by well twirling the hand holding the developing glass, pour off the water from the plate, and carefully pour on along the edge, or corner, this mixed fluid, so as to flow to the edges; rock as before; after a brief period, according to the appearance of the image, return the fluid to the developing glass and pour on again; repeat this several times, just holding the plate in the intervals between the eye and lamp to judge of the increased intensity, which, when it appears sufficient, should in the darkest parts permit the flame of the lamp to be just seen through them. Now wash well with water, finish with a little soft water; with a small towel wipe the back, and set the plate to drain in a plate rack, attaching to the lower corner a small piece of blotting paper, or the plate can be dried off at once over the lamp. It is sometimes difficult to judge of the real intensity gained under this treatment, when the image is observed by the light from the protected lamp, therefore, after the flowing over of the iodide of potassium solution, the remainder of the operations can be conducted by the direct light of the small lamp.

Should the development have been carried a little too far, or should the fine transparent markings appear thickened or clouded, before setting up the plate to drain, flush the plate with a mixture of equal parts of the cyanide and iodide solutions and distilled water, then well wash. Under this treatment many of the minute spots and half toned points become remarkably brightened. Some prefer to intensify before using the cyanide solution, by, first, under non-actinic light, after the iron developer has been *well* washed from the plate, pouring on the pyro solution, returning it to the developing glass, then adding the mixed silver solutions and repouring on and off the plate, until the image has been brought up to the necessary intensity, when it is to be well washed and then treated with the hypo fixing solution or the cyanide. These are returned to their vessels (short wide-mouthed

bottles or jugs are convenient), and can be used over and over again, adding fresh quantity as occasion may require; but in the case of the cyanide solution, it must not be left exposed, for it soon loses cyanogen, and the vapours are deleterious. Keep the hands continually wiped in these operations. If the plate, after the application of the iron solution and cyanide solution, have the appearance of under exposure, image indistinct in detail, or of being over exposed, image of a too dark and uniform character throughout, or of being out of focus, it will not be worth while to proceed to further develop it: wash it and carry it to the light, examine it with the hand magnifier, as some part, not that specially focussed, may appear the sharpest and serve to indicate the alteration required on re-focussing. If any extraneous light should have entered, through defects in the camera or at the vertical frame, or from the slide-holder, or when preparing the plate in the darkened closet, or before applying the fixing solutions, or if the nitrate bath and chemicals be not in perfect condition, the plate when cleared will appear fogged or misty, and not yield good prints.

Generally it is advisable, when the negative is good, to take a second one under the same arrangements, only re-arranging the prism; seldom can the exact relations be re-established, and after the rendering of another negative, it may be found that the little alteration in the illumination, not visible on the screen to the eye, has given a more perfect character to the image, or further developed some of the finer markings. It is also most convenient to follow up the operations, by taking photographs of the objects that are most suited for the present arrangement of the camera and lens.

**269. Varnishing the Plate.**—When the plates are dry, clean off the edges with a damp cloth held on the forefinger nail, wipe well the back, and hold the plate before a clear fire until moderately warm to the back of the hand; take it by one corner and pour on the varnish (Soehnée we employ). Allow it to flow freely over the surface and remain for half a minute or less on it, then pour back the surplus into the bottle from one corner, not rocking the plate; let it drain a little, then hold the plate towards the fire vertically, the edge from which the varnish was poured being downwards, and wipe this edge with a piece of rag to prevent a thickened line from being formed and extending inwards as the plate dries.



**270. Of Cleaning Old Plates.**—The soiled and used plates can be cleaned by the fresh use of washing soda; and those varnished can often be easily cleaned by being allowed to soak in a very hot strong solution of this substance, or rubbed with a pledget of tow dipped in nitric acid; but to be re-used they must be cleaned with great care.

**271. Of increasing the Intensity of the Negative.**—There is much difficulty to obtain a clean dense negative, that shall preserve distinctness in the finest markings, and when the attempt is made to procure greater intensity by the intensifying processes, the fresh deposit of silver, with the apparent shrinking of the collodion in drying, will often so completely close up these lines, that their definition becomes lost in the print. To endeavour to still preserve these and add printing intensity to the negative, some employ a solution of bichloride of mercury. Dissolve in distilled or soft water, 2 oz., 12 grains of the bichloride of mercury or corrosive sublimate; label the solution Poison. After developing with iron, washing and continuing the development with the silver and pyro solutions, fixing, and re-washing, the plate is flushed with the sublimate liquid (which is allowed to remain on, until the image becomes of a dark grey colour; if the solution be used weaker, 2 grs. to the oz. of water), then well washed, and recovered with a weak solution of iodide of potassium from 1 to 2 grs. to the oz. of water; this will give the image a dirty grey or green tinge, which will often dry of a darker colour. The bichloride can also be used after the iodide of potassium solution has been washed off, and after washing off with water, the plate may be covered with an old weak solution of hyposulphite of soda, or a few drops of the strong liquor of ammonia in half-a-pint of water. In cases in which the bichloride has been used to add to the intensity, the negatives, when dry, often present a remarkable sharpness; but it is no uncommon thing to find that when the plate has been dried, spontaneously even, the moment it is handled the collodion flies and cracks often into the image; to prevent this it is requisite to pour over the plate, after the last washing, a weak mucilage or gum water. In this case care must be taken to well dry the plate prior to varnishing, as gum is to a small extent an absorbent of moisture.

The third stage in the manipulation closes with the production of the image on paper, technically called Printing.



some size, into the first pour filtered rain water to the depth of half an inch, into the second a similar quantity of common water. Into a third dish, with deep sides, pour about a quart of water and half a drachm of the crystallized acetate of soda.

Remove the prints and float them face down on the water in the first dish,—when the surface is covered, gently tip the dish to wave the water across the printed surface without wetting the backs of the prints. After ten minutes lift them singly, and wipe across the wet side with a clean glass rod, then float them again on the water in the second dish; relay the first, and at the end of a like period treat the prints from the second dish as before, and push them into the water in the third dish—here keep them well stirred about and under the surface. In this they may remain half an hour. Some merely wash the prints in a large quantity of water.

**273. Toning Solution.**—The toning solution is prepared as follows:—

8 drachms of distilled water.

$7\frac{1}{2}$  grains of chloride of gold.

1 drop of hydrochloric acid is to be added to the above solution, which must be kept in a stoppered bottle in the dark.

The albumenized paper having been prepared, pour one drachm of the gold solution into a clean developing glass or measure, and add one ounce of distilled or soft water. Into another clean glass vessel put half an ounce of soft water, and 5 grains of bicarbonate of soda. Part of the soda solution is to be added to the gold gradually, stirring during the time. The solution is to be tested with blue litmus paper. The addition of soda solution is to be cautiously continued, until the paper is no longer reddened. A drop or two more of the soda is then to be added, and the neutralized solution of chloride of gold poured into a clean small flat dish, and mixed with about 8 ounces of soft water. Set this near to the window screened by the yellow curtain. Remove the washed prints from the third dish, wipe them back and front with the glass rod, and lean them against the inside walls of the dish to drain; then remove them and pass them into the toning solution. Here they are to be kept in motion: as they appear to darken, just lift the curtain aside and note the tint they have assumed by daylight, but they must not remain

exposed to the light any time, or the white parts will be injured. The other dishes should likewise be attended to and covered over with a sheet of paper to keep the light from them. When the prints appear to have the desired tint, from a warm brown, through neutral tint to nearly black, begin to remove the most toned, wipe them with the rod and pass them into a dish of clean water. The quantity of the toning solution prepared, must be in proportion to the size and number of prints.

The unaltered chloride of silver has now to be removed from the paper.

**274. Another Toning Solution.**—One grain of chloride of gold, or 1 drachm of the solution, is to be neutralized with bicarbonate of soda in 9 or 10 ounces of soft water, then half a drachm of the crystallized acetate of soda is to be added. This is to be used the day after making; it keeps well, and can be strengthened by adding freshly-made solution prepared somewhat stronger. Occasionally the neutral or alkaline solution of gold-bath will not act; but if the dish be set over a jug or basin of hot water, the toning action will commence, or a few drops of the chloride of gold may be added. Good toned prints have also been produced by using the weakened neutral solution of gold and soda for the next lot of prints, adding some fresh solution of gold. Other toning solutions are made with bichlorate, or phosphate of soda; also with chloride of lime. If the toning solution with acetate of soda be employed, it will not be necessary to use the acetate in the third dish of water. The object is to remove all the free nitrate of silver, that the gold bath may not be speedily decomposed.

**275. Fixing Solution.**—The fixing solution is made by putting into a gutta-percha dish, kept for this purpose only, according to the size and number of the prints,—

2 ounces of hyposulphite of soda to 8 or 10 ounces of soft water.

As a precaution, in case the hyposulphite should be acid, a small lump of chalk or whiting is to be added. Remove the prints from the water, drain well, if convenient, against the sides of the dish, then pass them singly into the fixing solution, keeping them there, in the case of a thin paper, for 10 minutes, and a thick paper for 15 minutes. They must be kept in motion. These

different processes should be conducted more or less continuously so as not to lose time.

When the prints are removed from the hyposulphite, drain well, then pass them into a vessel of clean water, which should be changed often during the first hour, draining completely each time. They may then be left for 6 hours, the water being changed perhaps every half hour. They are to be finished by soaking them for a short time in hot water. After this they are pinned up as before, and a piece of bibulous paper attached to the opposite end, so that the fluid may be drained off quickly. The hyposulphite solution should be used when freshly made.

**276. Of Mounting the Prints.**—The prints when dry are unpinned, pressed in a book or ironed on the back, then trimmed, and usually mounted on card, first laying them in the folds of a damp cloth, and the cards in another damp cloth. When the prints lie flat, they are to be removed to a clean surface of paper, and a stiff brush with thick mucilage from dextrine, or thick white starch paste, passed once over the back of the photograph, which is then placed on the card in the desired position. A clean fine cloth is passed over it, and the print pressed equally all over. Some use thin Scotch glue instead of mucilage.

When the cards are nearly dry, they should be passed through the rolling press.

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To enter more fully into the particulars connected with each branch of this study would be beyond the limits of this work. To those who desire more explicit information I must refer to the different works written on photography, especially to the excellent manual of my friend the Rev. Mr. Hardwich. Many useful hints will be found in the "*Photographic News Almanack*" for the current year. The appended list of references is, I believe, the most perfect which has yet been published; for this I have also to thank Dr. Maddox. In this department of microscopical work there remains much to be done, more particularly in reference to the use of artificial illumination and possibly the employment of the polariscope. This application of photography is somewhat limited, because it is not possible to obtain objects upon widely differing planes in focus at the same time, but if a proper selection of objects be made, far greater accuracy in the



delineation of minute parts will be gained than the draughtsman can possibly obtain.

*Photographs of Microscopic Objects for the Magic Lantern.*—Although no means are yet known by which a minute object magnified by the higher powers of the microscope can be thrown upon a screen so as to be seen by a number of persons at once, almost the same result has been obtained by magnifying the photograph of the object in an oxy-hydrogen magic lantern. Mr. Highley has been very successful in carrying out this object (see his paper read before the Society of Arts, January 14th, 1863).

As these photographs abound in delicate detail, an oxy-hydrogen or electric lantern with achromatic lenses is necessary for their proper display. The lantern and arrangements for producing the light are shown in Plate XLVII, Fig. 219. The lantern should be made of old seasoned mahogany, so that warping may not be produced by the very intense heat of the lime light. Behind the spring stage, which carries the photographic slide, is placed a combination of two lenses, 4 inches in diameter, called "the condenser," its office being to concentrate the light emitted by a cylinder of lime rendered incandescent by an ignited jet of oxy-hydrogen gas, upon the surface of the photograph, through which it passes, and then converges upon an achromatic combination placed at a proper focal distance in front. The rays on passing onwards diverge, and the enlarged shadow of the photograph is projected upon an opaque or transparent screen. By this means all the details of an object less than a pin's point in size may be shown with perfect definition, twenty feet in diameter. The hydrogen may be obtained from any house gas-supply by simply connecting the tap of a gas bracket by a piece of flexible tubing with the hydrogen tube of the jet. The oxygen is obtained by heating a mixture of chlorate of potash and oxide of manganese in a proper retort, and collecting the gas in a wedge-shaped gas-bag, after passing it through a washing bottle to purify it. The stopcock of the gas-bag is connected with the oxygen tube of the jet by flexible tubing. The jet is so arranged that it is impossible for any accident to occur in the shape of an explosion, the gases only being combined at the extremity of the jet.



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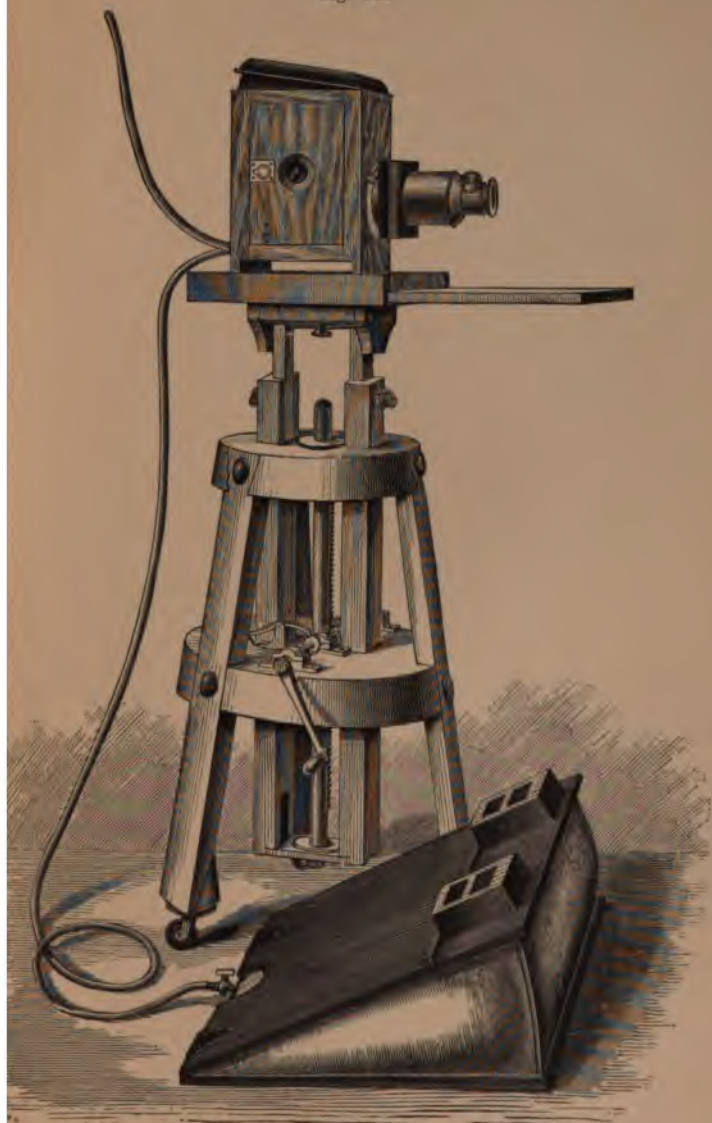
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Fig. 229.



§ 277.

Oxyhydrogen lantern, with gas bag, &c., as arranged by Mr. Highley for throwing the photographs of microscopic objects upon a screen.

[To face page 188.]



## CHAPTER X.

## NEW METHODS OF PREPARATION. OF STAINING TISSUES.

*—Process followed by the Rev. Lord S. G. Osborne—Gerlach's Method of Staining—Thiersch's Method—Thiersch's Lilac Colouring Fluid—Anilin Colours—Blue Colours for Staining—Tannic Acid—Solutions of Nitrate of Silver—Other Metallic Salts—Modification of the foregoing plans. THE AUTHOR'S NEW METHOD OF PREPARATION FOR EXAMINING TISSUES WITH THE HIGHEST POWERS.—Conditions to be fulfilled—Action of Glycerine and Syrup—The Injecting Fluid—The Carmine Fluid—Other Colouring Solutions—Glycerine and Acetic Acid—Chemical Reagents in Glycerine—Acetic Acid Syrup—Potash and Soda in Glycerine—Chromic Acid and Bichromate of Potash—Of the advantages of viscid media for the Dissection of Tissues—The practical method of preparing Tissues for examination with the Highest Powers—Of the preparation of Hard Tissues—New Theories relating to Structure and Growth arrived at by this mode of investigation.*

IN this Chapter I propose to give a brief account of some of the methods employed for staining tissues, and to describe in detail the special method I have myself found it necessary to adopt in carrying on investigations upon the structure of various textures with the highest powers of the microscope yet made. By this process, sections of every tissue may be prepared so that they may be examined by powers magnifying 2,000 diameters or more, and vessels may be injected and displayed in the same sections as other tissues.

This part of the subject, although of the greatest importance to the more advanced student, may, nevertheless, be studied by beginners who have honestly gone through the tables at the end of the volume. After the elementary principles have been mastered, the sooner the observer becomes familiar with the process of staining tissues, and the principles by which exceedingly thin sections may be obtained, the more quickly will he be in a condition to commence original research, and to improve upon the methods of investigation now in use.



## OF STAINING TISSUES.

The plan of staining tissues artificially, is one from which the greatest advantages have been derived already, and it is quite certain that, by modifications of the processes now employed, new and most important facts will be elucidated. This process of staining is one of the most valuable additions to our means of investigation that has ever been discovered. But it has not been sufficiently pointed out that the process of staining may be employed for very different purposes. 1. For staining the tissue or formed material. 2. For staining the nucleus or germinal matter. Neutral, acid, or alkaline fluids, will effect the first; but alkaline fluids, which possess considerable penetrating powers, are specially adapted for the second and most important object (*see my "Lectures on the structure and growth of the tissues," May, 1861*). An alkaline solution of carmine passes through the tissue, comes into contact with the germinal matter, by the acid of which it is decomposed, and the carmine is deposited so that it cannot be soaked out by the action of glycerine, as it may be from the matter of the tissue. Hence, in all cases, by taking certain precautions, we can always distinguish in any given tissue what parts are living and growing, and what parts have already been formed and have ceased to undergo vital changes. This matter is further considered in § 303.

**278. Process followed by the Rev. Lord S. G. Osborne.**—Welcker was, I believe, one of the first observers to employ a solution of carmine for the purpose of staining the nuclei of tissues, and Gerlach was an early and most successful advocate of this plan. It has been, but I think wrongly, stated, that Gerlach was the first who employed this process. The date of Gerlach's work is 1858 (*"Mikroskopische Studien aus dem Gebiete der Menschlichen Morphologie," Erlangen*). But in June, 1856, the Rev. Lord Osborne showed that nuclei were more deeply tinged by carmine than other parts of the cell. (*"Vegetable cell structure and its formation, as seen in the early stages of the growth of the wheat plant"*). See also the plate accompanying this paper (*"Trans. Mic. Soc.," Vol. v, Plate iv, 1856*). Lord Osborne allowed the plants to grow in the carmine solution. The growing parts were stained most successfully.

**279. Gerlach's Method of Staining.**—Gerlach used first a concentrated solution of carmine in ammonia, and placed the sections of brain and spinal cord previously hardened by chromic acid, to be tinged, in the carmine fluid for from ten to fifteen minutes. They were then well washed in water for some hours, and treated with acetic acid. The water and acid were removed by immersion in alcohol. The sections were afterwards mounted in Canada balsam. Gerlach found that dilute solutions (two or three drops of the ammoniacal solution of carmine to an ounce of water), and maceration for *two or three days*, afforded better results.

**280. Thiersch's Method.**—Frey ("*Das Mikroskop*") gives Thiersch's methods of colouring tissues by carmine. Some of them are as follows:—

Thiersch's red colouring fluid—

Carmine, 1 part.

Caustic ammonia, 1 part.

Distilled water, 3 parts.

This solution is to be filtered.

Oxalic acid, 1 part.

Distilled water, 22 parts.

One part of the carmine solution is to be mixed with 8 parts of the oxalic-acid solution, and 12 parts of absolute alcohol are to be added.

If the solution is orange-coloured instead of dark red, more ammonia is required, and the orange becomes red. The orange colour may also be used for staining. If crystals of oxalate of ammonia become formed they must be separated by filtration.

**281. Thiersch's Lilac Colouring Fluid.**—

Borax, 4 parts.

Distilled water, 56 parts.

Dissolve and add, of carmine, 1 part.

The red solution is to be mixed with twice its volume of absolute alcohol, and filtered. The precipitate of carmine and borax is re-dissolved in distilled water and is ready for use. It colours more slowly than the red solution.

**282. Anilin Colours.**—The beautiful reds and blues which have been lately so largely used as dyes, popularly known in this country as Magenta, Solferino, have been much employed by microscopists. The colour is not very soluble in water, but is readily dissolved by alcohol. A grain of the colour, ten or fifteen drops of alcohol, and an ounce of distilled water, make a dark red solution; or the colour may be boiled in water, allowed to cool, and then filtered. This fluid colours tissues very readily. Many exceedingly delicate and perfectly transparent textures, which are almost invisible in the natural state, can be most satisfactorily demonstrated by the use of this coloured fluid. The cilia of ciliated epithelium may be tinted while they continue to vibrate. As the substance of the cell becomes colored, however, the action of the cilia ceases.

**283. Blue Colours for Staining.**—Thiersch recommends the following fluid, the composition of which I take from Frey:—

Oxalic acid, 1 part.

Distilled water, 22 parts.

Indigo carmine, as much as the solution will take up.

Another solution of oxalic acid and water in the same proportion is required. One volume of the first solution is mixed with two volumes of the last and nine of absolute alcohol. The mixture is then filtered, and is ready for use.

An anilin blue fluid may be made as follows:—

Soluble anilin blue,  $\frac{1}{2}$  grain.

Distilled water, 1 ounce.

Alcohol, 25 drops.

This fluid is not acted upon by acids or alkalies. Frey strongly recommends a fluid of this description as very useful for colouring many tissues.

**284. Tannin.**—Although tannin does not colour animal membrane, it alters its character to such an extent as to enable us to see many peculiar points of structure or arrangement not visible before, or it produces a chemical change upon the substance, from which we gain important information. Solutions of magenta and solutions of tannin have been much used in investigations upon the blood corpuscles. The action of tannin

upon the red blood corpuscle is very peculiar; it has been specially studied by Dr. Roberts of Manchester ("On peculiar appearances exhibited by blood corpuscles under the influence of solutions of Magenta and Tannin"—"*Proceedings of the Royal Society*," Vol. XII, p. 481, No. 55, April, 1863). The solution is made by dissolving 3 grains of tannin in an ounce of distilled water. One drop of blood may be mixed with four or five drops of the tannin solution and a portion of the mixture examined under the microscope.

**285. Solutions of Nitrate of Silver.**—Of late years nitrate of silver has been used for staining tissues. Recklinghausen and His, have employed this plan with great success. For example, a weak solution may be imbibed by delicate tubes, and part being precipitated in the tube, perhaps as a chloride or in combination with some albuminous material, subsequently becomes decomposed by the action of light, and a very dark line results, and thus the position of a previously perfectly invisible channel is clearly demonstrated. The outlines of epithelial cells and the intervals between them may be demonstrated by this process. Transparent connective tissue and the outer part of cells can thus be coloured, the nuclei remaining perfectly colourless and transparent. The nuclei by longer immersion will also be coloured. The appearances may be made to vary very much by modifying the mode of procedure and the time which the preparation is allowed to remain in the solution. After soaking in the nitrate of silver solution for some time the specimen must be placed in distilled water, or in a weak solution of common salt, in order to wash away the nitrate which adheres to the surface or occupies the intervals between the cells. When this has been effected the specimen is exposed to daylight or sunlight until the requisite degree of blackening has been obtained. The strength of the solution employed may be varied according to circumstances. Recklinghausen uses a very dilute solution, consisting of 1 part of nitrate of silver to 400—800 of distilled water.

The structure of the cornea has been recently investigated by His, after the tissue was prepared according to this plan. The so-called 'intercellular substance' (formed material) only may be coloured, or, after the whole structure has been thoroughly impregnated with the solution, it may be soaked out



of the formed material, while that taken up by the nuclei (masses of germinal matter) is retained, and may be decomposed by being exposed to light. In this case the nuclei appear very dark and surrounded by a pale brown formed material. His thinks that when the nuclei are coloured, the precipitate of chloride of silver in the formed material is re-dissolved and absorbed by the nuclei, in which it is afterwards reduced by the action of the light.

**286. Other Metallic Salts.**—Tissues may also be impregnated with other solutions of metallic salts. Acetate of lead has often been employed. The tissue may be soaked for some time in a weak solution, or a weak solution with a little glycerine may be injected. When the tissues are well saturated, thin sections may be made, and, after being slightly washed, they may be placed in a dilute solution of glycerine, through which sulphuretted hydrogen may be passed. Living plants will take up solutions of various metallic salts, which may then be precipitated in the textures or in the channels by the appropriate reagents.

**287. Modification of the foregoing plans.**—The observer will perceive that these processes are capable of almost endless modification. Every one engaged in a special investigation, will naturally try various modes of preparation. Having decided upon one which seems to offer considerable advantages, he will try various modifications until he meets with success. I have not attempted to give the minute recommendations of various observers who have employed these processes; but merely indicate the general outline of the methods. A few experiments will teach the observer more than the most minute directions, and, however carefully directions may be given, it is seldom that anyone succeeds the first time he endeavours to follow them out. Those who desire to do real work in this department, must be patient, and must work on steadily until they meet with success. I propose now to describe the plan of investigation I have myself followed.

NEW METHOD OF PREPARATION ADAPTED FOR RESEARCHES  
WITH THE AID OF THE HIGHEST MAGNIFYING POWERS YET  
MADE.

For many years I have been strongly impressed with the notion, that advance in our knowledge of minute structure depended mainly upon improvements in the methods of demonstration. Experiments proved to me that it was not possible to make out the arrangement of the elements of the tissues of man and the higher animals in the recent state by examination in water, serum, vitreous humour, and other solutions usually employed for this purpose. In very many instances failure is due to the refractive power of the tissue and other physical characters. For instance, in the controversy now going on with reference to the arrangement of nerves in voluntary muscle, an independent witness would not fail to notice that very many different plans of demonstration had been employed by the various observers. This would, in some measure, enable him to explain the great discrepancy of the results. He would also notice that those who deny the facts stated by a previous writer, had not adopted the method of investigation recommended by him.

In my first paper upon the distribution of nerves and muscle, I stated that the facts I had demonstrated could not be seen unless a particular process of demonstration were followed, yet not one of my opponents has adopted the plan pursued by me, or considered the principles upon which its success depends. Nay, although I strongly insisted upon the importance of injecting the vessels, partly for the purpose of causing a preservative fluid to be distributed equally to all parts of the tissue, and partly to prevent the possibility of mistaking vessels for fine nerve fibres, not one of my opponents, I believe, has yet injected the vessels. The statements I have made upon the mode of preparation, result from numerous experiments made during the last twelve years. Many of my opponents endeavoured to throw doubts upon my results by describing the little they have themselves been able to see by the rough processes they have followed. But it need scarcely be said, that the stout denial often given to the existence of a particular arrangement, really means only that the individual who denies has never seen it. The only wonder is, that any anatomist should be so blind as to persuade himself that

he has seen all that can be seen. It has been said that other observers have not met with the success, in preparing specimens of the liver demonstrating the continuity between the ducts and cell-containing network, which has attended my efforts. The details of that process have been given, and if the principles laid down be acted upon, it is, I believe, impossible for anyone with ordinary dexterity and moderate patience to fail.

I cannot venture to hope that many facts I have observed in the minute structure of the central and peripheral nervous system, will be confirmed until the same process adopted by me is followed by others. It is true that the specimens can be shown to others: but it so happens that working men have but few opportunities of examining each other's specimens, and when an opportunity does occur, it not unfrequently happens that time is not allowed to investigate the specimens fairly. The consequence of all this is, that working in circles goes on to a terrible extent. Great labour is utterly wasted, and there is but very slow progress compared to that which would attend our efforts if observers generally were agreed upon the principles upon which anatomical observations should be conducted. Doubtless, every observer soon finds out valuable methods of detail for himself which satisfy him,—but as will not be able in many cases to communicate to others the practical manipulations upon which his success depends, it is often exceedingly difficult to ascertain the real merits of any given process. Still, it is a question capable of being settled most positively, whether nerves can be followed in tissues which are impregnated with syrup, glycerine, or some such medium, for a greater distance than when immersed in water, serum, vitreous, &c., and whether or not more fibres and finer fibres can be seen in the former than in the latter case. A simple experiment will convince anyone that this is so, and if observers would prepare small portions of the same tissue in these two different media, and compare the results, they would, I am sure, soon be agreed upon one principle of great importance in investigation. It is mainly with the view of encouraging free discussion upon this most important question, and in the hope that ere long some general process of investigation may be followed, that I publish my own conclusions and describe somewhat minutely the process which I now follow. I do not for one moment pretend that it is equally applicable to all tissues, or that it will succeed in all hands; but I am



confident that it is based upon principles of the utmost importance for successful demonstration. Every year I myself discover improvements in detail of the utmost advantage; but the basis of the process remains the same, and, as I have now been actively engaged in minute microscopical investigation for fifteen years, it is scarcely possible that a process which has been adhered to so long, can be destitute of advantages over many other methods. Moreover, in the hands of some of my pupils it has answered as well as in my own.

**288. Conditions to be fulfilled in Demonstrating Minute Structure by the Highest Powers.**—In order to demonstrate the intimate structure of most soft tissues, very high magnifying powers are required, and it is important to consider the following points:—

1. Of many tissues, sections sufficiently thin for high powers cannot be obtained by the processes usually adopted. In order to make the specimen thin enough, pressure must be employed, and in many instances very strong pressure is required. Even by very moderate pressure, tissues immersed in water are destroyed completely, and experience has proved that the requisite amount of pressure can only be employed if the tissue be immersed in, and thoroughly impregnated with, a viscid medium, which is not only readily miscible with water in all proportions, but with such chemical reagents as may be required to act upon one or more constituents of the tissue for the purposes of demonstration.

2. As many structures are exceedingly delicate, and undergo change very soon after death, it is necessary that the medium in which they are examined should have the property of preventing softening and disintegration, and should act the part of a preservative fluid.

3. In order that tissues should be uniformly permeated with a fluid within a very short time after the death of the animal, it is necessary that the fluid should come quickly in contact with every part of the texture. This may be effected in two ways:—

- a.* By soaking very thin pieces in the fluid, or

- b.* By injecting the fluid into the vessels of the animal.

4. As different structures require fluids of different refractive power for their demonstration, the medium employed must be



such that its refractive power can be increased or diminished, or that, for the medium fulfilling the former condition, another can be readily substituted which fulfils the latter requirements.

5. In investigations upon the changes which structure undergoes in the organism, it is necessary to distinguish between that part of the texture which is the oldest, and that which has just been produced—between matter in which active changes are going on, and matter which is in a passive state. It is only by fulfilling this requirement that the direction in which growth takes place, and the point where new matter is added, can be ascertained.

6. It is necessary, in many investigations, that the vessels should be positively distinguished from the other constituents of the tissue, and it is necessary that the process by which this is effected, should not interfere with the demonstration of all the tissues in the immediate vicinity of the vessels.

7. It is of the utmost importance that the medium employed for demonstration should have the property of preserving the specimens, so that observers should be able to exhibit their preparations to others.

Glycerine and syrup fulfil the requirements mentioned in the foregoing paragraphs.

**289. Action of Glycerine and Syrup on Tissues.**—Strong syrup may be made by dissolving, with the aid of heat, lump sugar in distilled water, in the proportion of about three pounds to a pint. It is necessary in many cases to employ the strongest glycerine. In this country we have had the advantage of the beautiful preparation called Price's glycerine, which is made of specific gravity 1240. It has been said that glycerine and strong syrup are not adapted for preserving soft tissues, because the tissues shrink and soft cells collapse in consequence of exosmose of their fluid contents. But I have many hundred specimens preserved in the strongest glycerine I could procure, and I should obtain advantages if glycerine could be made of still greater density. There would be no difficulty in impregnating even very soft tissues with it. In fact, the objections urged are theoretical, and result from ignorance of some properties of the tissues on the part of those who have urged them. If objectors had simply tried the experiment, they would have found no difficulty what-

ever in the process. The fact is, that tissues possess a considerable elastic property, and although they shrink when immersed in a medium of considerable density, they gradually regain their original volume if *left in it for some time*. In practice, the specimen is first immersed in weak glycerine or syrup, and the density of the fluid is gradually increased. In this way, in the course of two or three days, the softest and most delicate tissues may be made to swell out almost to their original volume. They become more transparent, but no chemical alteration is produced, and the addition of water will at any time cause the specimen to assume its ordinary characters.

The hardest textures, like bone and teeth, may be thoroughly impregnated and preserved in strong glycerine. Cerebral tissues, delicate nervous textures like the retina, or the nerve textures of the internal ear, may be permeated by the strongest glycerine, and when fully saturated with it, dissection may be carried to a degree of minuteness which I have found impossible in any other medium. Nor is the use of glycerine and syrup confined to the tissues of man and the higher animals. I have preparations from creatures of every class. The smallest animalcules, tissues of entozoa, polyps, star fishes, mollusks, insects, crustacea, various vegetable tissues, microscopic fungi and algae of the most minute and delicate structure, as well as the most delicate parts of higher vegetable tissues, may all be preserved in these viscid media; so also may be preserved the slowest and most rapidly growing, the hardest and softest morbid growths, as well as embryonic structures at every period of development, even when in the softest state. I am, indeed, not acquainted with any animal or vegetable tissues which cannot with the greatest advantage be mounted thus. All that is required is, that the strength of the fluid should be increased very gradually until the whole tissue is thoroughly penetrated by the strongest that can be obtained. Glycerine has long been in use among microscopists, but my object is to show that it is universally applicable, that it or syrup may be made the basis of all solutions employed by the microscopical observer with the greatest advantage, that many points are to be demonstrated by the use of these solutions, which have hitherto escaped observation, and that there are reasons for believing that very much may yet be discovered by the use of these substances.

From these general remarks, I pass on to describe, more in

detail, the particular method I have adopted during the last four years for minute investigations upon structures magnified by the highest powers yet employed. It will be necessary, in the first place, to give the composition of the different solutions which I find useful for general purposes.

**290. Glycerine and Syrup.**

1. *Weak common glycerine* of about the specific gravity 1050.
2. *The strongest Price's glycerine* that can be obtained.
3. *Syrup* made by dissolving, by the application of a gentle heat in a water bath, 3 lbs. of sugar in a pint of distilled water. A weaker solution can be prepared, as required, by mixing equal parts of syrup and water.

**291. The Injecting Fluid.**—For the purposes of injection I have found a slight modification of the original Prussian blue fluid, the composition of which is given in § 196, fulfil all the requirements. The following mixture has succeeded admirably in my hands, and I therefore, recommend it strongly. It penetrates to the finest vessels. It never forms a deposit. The specimens injected with it retain their colour perfectly, and the injected tissues can also be stained with carmine.

Price's glycerine, 2 oz. by measure.  
Tincture of sesquichloride of iron, 10 drops.  
Ferrocyanide of potassium, 3 grains.  
Strong hydrochloric acid, 3 drops.  
Water, 1 oz.

Mix the tincture of iron with one ounce of the glycerine ; and the ferrocyanide of potassium, first dissolved in a little water, with the other ounce. These solutions are to be mixed together very gradually in a bottle, and are to be well shaken during admixture. The iron solution must be added to the ferrocyanide of potassium. Lastly, the water and hydrochloric acid are to be added. Sometimes I add a little alcohol (2 drachms) to the above mixture.

This fluid does not deposit any sediment, even if kept for sometime, and it appears like a blue solution when examined under high magnifying powers, in consequence of the insoluble particles of Prussian blue being so very minute.



292. The Carmine Fluid.—The following is the composition of the carmine fluid which I use :—

Carmine, 10 grains.  
Strong liquor ammonia,  $\frac{1}{2}$  drachm.  
Price's glycerine, 2 ounces.  
Distilled water, 2 ounces.  
Alcohol,  $\frac{1}{2}$  ounce.

The carmine in small fragments is to be placed in a test tube, and the ammonia added to it. By agitation, and with the aid of the heat of a spirit-lamp, the carmine is soon dissolved. The ammoniacal solution is to be boiled for a few seconds and then allowed to cool. After the lapse of an hour, much of the excess of ammonia will have escaped. The glycerine and water may then be added and the whole passed through a filter or allowed to stand for some time, and the perfectly clear supernatant fluid poured off and kept for use. This solution will keep for months, but sometimes a little carmine is deposited, owing to the escape of ammonia, in which case one or two drops of liquor ammonia to the four ounces of carmine solution may be added.

The rapidity with which the coloring of a tissue immersed in this fluid takes place, depends partly upon the character of the tissue and partly upon the excess of ammonia present in the solution. If the solution be very alkaline the coloring is too intense, and much of the soft *tissue* or imperfectly developed formed material around the germinal matter, is destroyed by the action of the alkali. If, on the other hand, the reaction of the solution be neutral, the uniform staining of tissue and germinal matter may result, and the appearances from which so much is learnt are not produced. When the vessels are injected with the Prussian blue fluid the carmine fluid requires to be sufficiently alkaline to neutralise the free acid present. The permeating power of the solution is easily increased by the addition of a little more water and alcohol.

Some tissues are colored very slowly. Fibrous tissue, bone and cartilage, even in very thin sections, will require twelve hours or even more, but perfectly fresh soft embryonic tissues, and very thin sections of the liver and kidney, thin sections of morbid growths rich in cells, may be colored in half an hour, while the cells of the above structures, placed on a glass slide, may be colored in less than a minute. I have often colored the germinal



I keep various tests, such as alcohol, ether, the various acids and alkalies, and other tests in the form of viscid solutions made with glycerine or sugar. The reaction of the iodine tests for amyloid matter, starch and cellulose, is much more distinct when employed in this manner. The plan is, to allow the texture to be tested to be thoroughly saturated with the strong<sup>\*</sup> glycerine solutions, and then to add water. In the course of a few hours the reaction takes place very strongly.

299. *Of the Advantages of Viscid Media for the Dissection of Tissues for Examination with the Highest Powers.*—I carry on minute dissection in these viscid media, and can readily detach the most minute parts of tissues, separate the different structures in one texture, without tearing or destroying them, unravel convoluted tubes, and perform with ease a great variety of minute operations, which it would be impossible to effect by any of the ordinary methods of dissection. With care in regulating the temperature, I can soften textures thus preserved in syrup to the precise extent required for further minute dissection, and even very hard textures may thus be softened, so that by gradually increased pressure and careful manipulation, exceedingly thin layers can be obtained without the relation of the anatomical elements to each other being much altered, and without any of the tissues being destroyed.

300. *The Practical Operation of Preparing Tissues for Examination with the Highest Powers.*—The general plan I follow, is the same for all tissues of all vertebrate animals and morbid growths; but I will describe the several steps of the process as they were conducted in the demonstration of the structure of the ganglion cells, described in my paper in the "*Phil. Trans.*" for 1863,<sup>\*</sup> and of the structure of the papillæ of the frog's tongue, described in the communication presented to the Royal Society in June of the present year.<sup>†</sup>

The description given also applies to the mode of preparing specimens of muscular fibre to demonstrate the mode of distri-

\* "On the structure and formation of the so-called apolar, unipolar, and bipolar nerve cells of the frog." May, 1863.

† "New observations upon the minute anatomy of the papillæ of the frog's tongue." June, 1864.

bution of the finest branches of nerve fibre.\* It is the same plan which I have followed in the investigation of the minute structure of the brain, spinal cord, and ganglia of man and the higher animals.†

My researches upon the tissues of the frog have been principally conducted upon the little green tree frog (*Hyla arborea*), for experience has proved to me that the tissues of this little animal are so much more favourable for investigation than those of the common frog, that it is well worth while to obtain specimens, even at the cost of 2s. or 2s. 6d. each.

The frog is killed by being dashed suddenly upon the floor, but it must first be carefully folded up in the centre of a large cloth, so that the tissues may not be bruised in the least degree. Next an opening is made in the sternum, the heart exposed, and a fine injecting pipe, after being filled with a little injection, is tied in the artery. This part of the operation is conducted as fully described upon page 118, except that the Prussian blue fluid given in § 291, is used instead of the more inexpensive fluid made with common glycerine. The injection ought to be complete in from twenty minutes to half an hour, and sometimes in less time than this. The injection, being pale, cannot be very distinctly seen by the unaided eye, but if the operation has been conducted successfully, the tissues will be found swollen and the areolar tissue about the neck will be fully distended. The observer must not attempt to inject a *Hyla* before he has succeeded in injecting the common frog perfectly, for the *Hyla*, being smaller, is somewhat more difficult to inject than the common frog or the newt.

The injection being complete, the abdominal cavity of the frog is opened, and the viscera washed with strong glycerine. The legs may be removed, the mouth slit open upon one side, and the

\* On the distribution of nerves to the elementary fibres of striped muscle. "*Phil. Trans.*," 1860.

Further observations. "*Phil. Trans.*," 1862.

Further observations in favour of the view that nerve fibres never end in voluntary muscles. "*Proceedings of the Royal Society*," June 5th, 1863.

On the structure of the sarcolemma of the muscular fibres of insects, and of the exact relation the nerves and tracheæ to the contractile tissue of muscle. "*Microscopical Society*," June, 1864.

† On the minute structure of the grey matter of the convolutions of the brain. "*Proceedings of the Royal Society*," Vol. xii, 671, 1863.

Indications of the paths taken by the nerve currents as they traverse the caudate nerve cells of the spinal cord and encephalon. "*Proceedings of the Royal Society*," July, 1864.

pharynx well washed with glycerine. If it is desired to prepare one organ only, this may, of course, be removed and operated upon separately; but I generally subject the entire trunk, with all the viscera, to the action of the carmine fluid. If the brain and spinal cord are special objects of inquiry, the cranium and the spinal canal must be opened so as to expose the organs completely, before the staining process is commenced. Enough of the carmine solution is then placed in a little porcelain basin or gallypot, just sufficient to cover the entire trunk and viscera. The specimen is then moved about in the carmine fluid, so that every part that is exposed is thoroughly wetted by it; sometimes slight pressure with the finger is required. It is left in the carmine fluid for a period varying from four to six or eight hours, being occasionally pressed and moved about during this time, so as to ensure the carmine fluid coming into contact with every part. By this time the blue colour of the vessels of the lungs, viscera, &c., will have almost entirely disappeared, and all the tissues will appear uniformly red. The staining is now complete. The carmine fluid is poured off and thrown away, and the preparation washed quickly with the glycerine solution (page 200). This fluid may be placed in a wash bottle, made according to the plan figured in 'Plate XXXVII, Fig. 173, but smaller than this, and projected upon every part so as to wash away the superfluous carmine fluid. The specimen is now placed in another little basin, and some strong glycerine poured over it; it is then left for two or three hours, and a little more strong glycerine added; when, from six to twelve hours since the specimen was removed from the carmine solution have elapsed, the preparation is ready for the last preliminary operation. The glycerine used for washing it is poured off, and sufficient strong Price's glycerine added just to cover it. To this, three or four drops of strong acetic acid are added, and well mixed with the glycerine. In this acid fluid the preparation may be left for several days, when a small piece of some vascular part may be cut off, placed in a drop of glycerine, and subjected to microscopical examination. If the injected vessels are of a bright blue colour, and the nuclei of the tissues of a bright red, the specimen is ready for minute examination; but if the blue colour is not distinct, three or four more drops of acetic acid must be added to the glycerine, and the preparation soaked for a few days longer.

If the nuclei are of a dark red colour, and appear smooth and



homogeneous, more especially if the tissue intervening between them is coloured red, the specimen has been soaked too long in the carmine fluid; but in this case, although parts upon the surface may be useless for further investigation, the tissues below may have received the proper amount of colour.

The tissues or organs to be subjected to special investigation may now be removed, and transferred to fresh glycerine; they may be kept in little corked glass tubes, and properly labelled. Generally, the tissue will contain sufficient acetic acid, but if this is not the case, one drop more may be added.

Suppose, now, the nerves with the small vessels and areolar tissue at the posterior and lower part of the abdominal cavity have been placed in one tube, and the prepared tongue of the *Hyla* in another, the former specimen may be taken out of the glycerine and spread out upon a glass slide. If it be examined with an inch power, numerous microscopic ganglia may be seen. Several of these perhaps are close to small arteries. Those which are most free from pigment cells are selected, and removed carefully by the aid of a sharp knife, fine scissors, forceps, and a needle point. This operation may be effected while the slide is placed upon the stage of the microscope. The *transmitted light* enables the observer to see the minute pieces very distinctly; if necessary, a watchmaker's lens may be used. The pieces selected are transferred to a few drops of the strongest glycerine placed in a watch glass or in one of the little china colour moulds (§ 85), and left to soak for several hours.

The microscopical examination of the specimen may now be carried out. One of the small pieces is placed upon a glass slide, in a drop of fresh glycerine, and covered with thin glass. The glass slide may be gently warmed over the lamp, and the thin glass pressed down upon the preparation by slight taps with a needle point. The specimen may now be examined with a quarter, and afterwards with the twelfth of an inch object-glass. A good deal of granular matter will possibly obscure the delicate points in the structure. The slide is again gently warmed, and, with the aid of a needle, the thin glass is made to slide over the surface of the specimen, without the position of the latter being altered, and then removed and cleaned. The specimen is then washed by the addition of drop after drop of strong glycerine containing five drops of acetic acid to the ounce. The slide can be slightly inclined while it is warmed gently over the lamp, in such a



manner that the drops of glycerine slowly pass over the specimen and wash away the debris from its surface. The most convenient instrument for dropping the glycerine on the specimen is a little bottle, of two ounces capacity, with a syphon tube drawn to a point and a straight tube, with an expanded upper part, over which is a piece of stout sheet vulcanized India-rubber. Fig. on p. 10. Upon compressing the air, by pressing down the India-rubber, glycerine is forced drop by drop through the syphon tube and allowed to fall upon the specimen. These little bottles can be obtained of Mr. Matthews, Carey-street, Lincoln's inn-fields.

When several drops of pure glycerine have been allowed to flow over the specimen, the thin glass cover, after having been cleaned, is re-applied and pressed upon the specimen gradually, but more firmly than before. If the preparation is now pretty clear when examined with the twelfth, the glass cover may be cemented down with Bell's cement, and the specimen may be left for many days in a quiet place. It may then be re-examined, and the process of washing with glycerine repeated, and further pressure applied until it is rendered as thin as is desired. When this point has been reached, more glycerine with acetic acid is to be added, and a plate of mica or the *thinnest glass cover* (§ 3) is applied, when it may be examined with the twenty-fifth. The process of flattening may be pushed still further if desirable,—if only carried out very slowly by gentle taps or careful pressure with the finger and thumb, *from day to day*, the elements of the tissues are gradually separated without being destroyed. If there be much connective tissue, which interferes with a clear view of the finest nerve or muscular fibres, it may be necessary to immerse the specimen for some days in the acetic acid syrup, and then transfer it to fresh glycerine. The success of this process depends upon the care and patience with which it is carried out. The most perfect results are obtained in cases where the washing with pressure, and warming have been very slowly conducted, and it is most interesting to notice the minute points of structure which are gradually rendered clearer by the application of a gentle heat, subjecting the specimen to a little firmer pressure or soaking it in a little fresh glycerine placed in a watch-glass.

Specimens of tissue prepared in this way can be transferred from slide to slide, and no matter how thin they may be, after having been allowed to soak in fresh glycerine they may always be laid out again perfectly flat by the aid of needles upon another

slide.\* The action of these viscid fluids is most valuable, and I feel sure that by the process here given, retaining the principle, but modifying the details in special cases, many new and important anatomical facts will be discovered. Until this process is carried out successfully by other observers, I have little hope of my own observations being confirmed.

The papillæ of the frog's tongue are prepared in precisely the same way. Small pieces of the mucous membrane being removed by sharp scissors, they are transferred to glycerine, subjected to the same very gradually increased pressure, until the individual papillæ are themselves slightly flattened. It is possible from a specimen to remove a number of the separate papillæ on a needle point, transfer them to glycerine or to the acetic acid syrup, and then mount them for examination with the  $\frac{3}{4}$ th object-glass. All the points I have described and figured in my paper ("Royal Society, 1864") may then be demonstrated in several papillæ.

Thin sections of brain, spinal cord, &c., may be subjected to the same process for examination with the highest powers. The specimens illustrating my paper on 'Indications of the paths taken by the nerve currents as they traverse the caudate nerve cells of the spinal cord and encephalon,' published in the "*Proceedings of the Royal Society*," July, 1864, were prepared in the manner already described, but they were soaked for some months in a weak glycerine solution of acetic acid. The most delicate preparations retain their characters for many months, and some for several years, so that in many cases the very preparations from which my drawings have been made, have been preserved, and have been compared with the drawings by other observers.

**301. Of the Preparation of Hard Tissues for examination with the Highest Powers, Bone, Teeth, &c.**—The methods generally employed (§ 149) for demonstrating the structure of bone, teeth, and other hard tissues, only enable us to form a notion of the dead and dried tissue. The soft material is dried up before the section is made.

\* I often mount these specimens upon a circle of thin glass about  $\frac{1}{4}$  of an inch in diameter, instead of upon a glass slide. The circle is then mounted upon a wooden slide, in the centre of which a hole has been drilled of the proper dimensions to receive the circle. It is fixed in its place by a ring of gummed paper.

And yet this very soft material, which is not represented in the drawings published in different works, is that which makes the only difference between the dried bone or tooth in our cabinets and that which still remains an integral part of the living body. So far from this soft matter being unimportant, it is the most important of all the structures of the hard texture. It is by this alone that all osseous and dental tissues are formed and nourished, and from not recognising the arrangement of this soft matter the most erroneous ideas have prevailed, and still prevail, upon the formation and nutrition of the dental tissues.

Even now it is generally believed that the dentinal tubes are real tubular passages for conveying fluids to all parts of the dentine, and are thus subservient to its "nutrition," and yet it is more than eight years since Mr. Tomes proved most conclusively that these so-called "tubes" were occupied in the recent state by a moist but tolerably firm material (*"Phil. Trans.,"* Feb., 1856).

I have verified Mr. Tomes' description, and am quite certain that the so-called dentinal tubes are not channels for the mere flowing up and down of nutrient fluid.\*

Suppose a tooth is to be prepared for minute microscopical investigation, we may proceed as follows. The same plan is applicable to bone and shell.

1. As soon as possible after extraction, the tooth may be broken by a hammer into fragments, so as to expose clean surfaces of the tissues. Pieces of dentine with portions of pulp still adhering to them may then be selected and immersed in the carmine fluid, and placed in a vessel lightly covered with paper, so as to exclude the dust. The whole may be left in a warm room for from twenty-four to forty-eight hours.

2. The carmine solution may then be poured off, and a little plain dilute glycerine added. (§ 294.)

3. After the fragments of teeth have remained in this fluid for five or six hours, the excess, now coloured with the carmine, may be poured off, and replaced by a little strong glycerine and acetic acid. (§ 294.)

4. After having remained in this fluid for three or four days, it will be found that the portions of soft pulp have regained the

\* On the structure of recent bone and teeth, see my lectures on "*The structure and growth of the tissues.*" Royal College of Physicians, 1860.



volume they occupied when fresh. They have swollen out again even in the strongest glycerine.

5. I have found that in many cases, when it is desired to study the arrangement of the nerves, it is necessary to harden the pulp by immersion in glycerine solution, made by adding to an ounce of the glycerine solution of acetic acid, two or three drops of a strong solution of chromic acid. The fragments may remain in this solution for three or four days, and then be transferred to the acetic acid solution, in which they may be preserved for years with all the soft parts perfect.

6. The specimens are now ready for examination. Thin sections are *cut* with a knife from the fractured surfaces of the dentine, including a portion of the soft pulp. The knife should be strong, but sharp. In practice I have found the double-edged scalpels made for me by Messrs. Weiss and Son, of the Strand, answer exceedingly well for this purpose, nor will the edge of the knife be destroyed so soon as would be supposed.

7. The minute fragments of sections thus obtained are placed upon a slide and immersed in a drop of pure strong glycerine, in which they may be allowed to soak for an hour or more, and then examined by a low power (an inch). The best pieces are then to be selected by the aid of a fine needle, and removed to a drop of glycerine containing two drops of acetic acid to the ounce, and placed upon a clean slide. The thin glass cover is then carefully applied, and the specimen may be examined with higher powers.

8. If it is desired to retain the specimen, the excess of glycerine fluid is absorbed by small pieces of blotting-paper, and the glass cover cemented to the slide by carefully painting a narrow ring of Bell's microscope cement (§ 90) round it. When this first thin layer is dry, the brush may be carried round a second time, and after the lapse of a few days, more may be applied. Mounted in this way the specimen will retain its character for years.

Hard tissues, like bone, dentine, and enamel, become somewhat softened by prolonged maceration in glycerine, and if a few drops of acetic acid are added, the softening process may be carried to a greater extent, and yet without the calcareous matter being dissolved out to any perceptible extent. If desired, of course the calcareous matter may be in part or entirely removed by increasing the strength of the acid fluid in which the preparation is immersed. But, far short of this, the hard, brittle texture is so altered that thin sections may be *cut* without any difficulty.



Specimens prepared in this way may be examined by the highest magnifying powers yet made, by which statement I mean, of course, to imply that more may be learned by the use of such high powers (1,000 to 3,000 linear) than by employing ordinary object glasses.

**302. The Preparation of Embryonic Tissues for Examination with very High Powers.**—Contrary to general opinion, many of the softest textures may be investigated with the greatest facility after having been soaked in strong glycerine. In preparing these, the same steps which have been described in § 300 must be carried out, but the glycerine used at first must be weaker, and its strength must be very slowly and gradually increased. Young embryos may be injected with the Prussian blue fluid. The pipe cannot be tied in the vessels, as they are extremely soft. But if it is simply inserted, much of the injection will run onwards into the capillaries, and the escape of a certain quantity by the side of the pipe is a matter of no moment.

I have beautiful preparations of the most delicate embryonic tissues, preserved in the strongest glycerine. It is often advantageous to harden the tissue slightly by the addition of a little of the chromic acid glycerine solution. When once the tissues have been fully permeated by glycerine, they may be dissected and manipulated in a manner which before was impossible.

**303. New Views relating to Structure and Growth arrived at by this mode of investigation.**—The general inferences arrived at from a careful study and comparison of very many animal and vegetable tissues, prepared by precisely the same process, are of great interest. When the carmine fluid is used properly, the so-called nucleus is alone coloured, while the outer part of the cell and intercellular substance remain colourless, or are only tinted very faintly. It is often possible to demonstrate zones of color one within the other, the innermost being invariably colored most intensely.

By comparative observations upon the same tissues, at different periods of growth, a definite but gradually altering relation has been demonstrated to exist between the formed material and the germinal matter, and it has been proved that the latter gradually passes into the former. I have adduced very many facts, which seem to me to establish the very important point,

that all formed material was once in the state of germinal matter. So that in the formation of muscle, for example, from the lifeless nutrient pabulum in the blood, the matter which is to become muscle passes through these different conditions:—

1. Soluble nutrient matter, or pabulum.
2. Germinal matter (nucleus).
3. Imperfectly developed formed material, or muscular tissue.
4. Fully developed formed material, muscular contractile tissue.
5. Disintegrated formed material, which becomes reduced to a soluble state, and converted, by oxydation, into new substances, some of which pass away, while others in their turn become pabulum for other kinds of germinal matter (white blood corpuscles, lymph corpuscles?).

It will be seen that one very important fact gained by this inquiry, is the positive distinction between the *active living growing matter* of all tissues, and the matter which is *formed*, or *results from the changes occurring in the former*. This fact I endeavoured to establish in my lectures, given at the Royal College of Physicians, in April, 1861. Since this time I have worked out the growth and formation of many tissues in detail, and I believe the above positions have been fully established. So that the material stained by carmine is in a transition state. It is not *tissue*, but it lives and grows, and at length undergoes conversion into tissue. It is living matter;—and by the word *living*, I mean, that in this matter phenomena are observed which have not been explained, which cannot be accounted for by any known laws, which cannot be imitated artificially, and which have never been observed anywhere but among living things.\* Among the peculiar properties of every mass of living matter, are—

1. The power of altering and appropriating certain soluble matters, and communicating to these, properties or powers of the same nature as those which the living matter itself possesses.
2. The power of moving in all directions—the passage of one part of a living mass to another part, so that one portion may advance *itself* in front of another portion, or encircle another.
3. The power of causing the elements of which the matter itself consists to take up definite relations towards one another,

\* This question is discussed in some papers published in "*The Reader*," in 1864.

so that definite compounds, often exhibiting definite structure, result.

#### 4. The power of infinite increase.

I am, therefore, able to describe the structure of the most complex tissues, and the changes which occur during their growth, in a very simple manner. It is not necessary to discuss in any given case what is 'cell wall,' or 'cell membrane,' 'cell contents,' 'nucleus,' 'nucleolus,' 'intercellular substance,' 'primordial utricle,' 'protoplasm,' 'Blastema.' For every structure consists of matter in two states:—*The living or germinal state, and the formed and lifeless state.* All increase, multiplication division, &c., is due to matter in the first state, and to that alone; so that every living particle comes from a pre-existing living particle, and every piece of tissue, and formed matter of every kind characteristic of a living being, was once in the condition of germinal matter.

These views will be understood by reference to Plates L, LI, LII, LIII, LIV, LV, and the accompanying explanations at the end of Chapter XI. The illustrations are the result of great labour, and are examples of the best kind of wood engraving and printing. Some have been selected from drawings illustrating my papers in the "*Phil. Trans.*," and I am indebted to the Council of the Royal Society for permission to make use of some of them.

NOTE to § 300.—I should have stated at the end of § 300, that I have prepared many specimens by injecting the colouring carmine fluid into



This fig. shows the manner of constructing the little bottle for containing glycerine, referred to in page 208.

the vessels, allowing time for this to be taken up by the nuclei, and then injecting an acid fluid; for example, a frog may be injected with the carmine fluid, made as recommended on p. 201, but with 15 grains of carmine instead of only 10. When the vessels are fully distended, the preparation is left for from 12 to 24 hours, when a little glycerine may be forced in, and lastly, the Prussian blue injecting fluid. When the vessels are fully injected, pieces of the tissue and organs to be examined are to be soaked in glycerine and acetic acid, as recommended in the other mode of preparation. I have some very beautiful specimens prepared according to this plan.



## CHAPTER XI.

OF THE USE OF VERY HIGH MAGNIFYING POWERS.—  
*Objections raised—Of the Highest Magnifying Powers yet  
 made—Of the Covering Glass—Illumination of Objects  
 Magnified by very High Powers—Method of Increasing  
 the Size of the Image without altering the Object-glass—  
 Of Drawing Objects Magnified with very High Powers.*  
 OF THE ANATOMICAL ELEMENT OR CELL, AND OF ITS  
 LIFE.

304. **Objections raised to the use of very high Magnifying Powers.**—The greatest prejudice still exists in the minds of many against the use of very high magnifying powers, and some persons still persist that no advantage is to be gained by them. Now, it would be wasted purpose for me to attempt to answer the objections that have been raised to this and other methods of observation, in Germany and elsewhere. Every observer has a right to work as he likes, and it is impossible to prevent prejudiced persons from disparaging the means of research which they cannot or will not employ. Just as there are observers who will not admit that the simplest and only efficient manner of introducing fluid into all parts of a tissue is to inject it by the vessels, so there are individuals who will maintain that those appearances can alone be trusted, and accepted, as natural appearances, which result from observations upon tissues immersed in water. And as it is most certainly true, that nothing is gained by subjecting specimens immersed in water to the highest powers, no wonder authorities who hold this doctrine assert that high powers are useless. But it has been proved that water alters many tissues extremely, and completely destroys some of the most delicate textures, while its limpid character renders it impossible to fray out many delicate tissues, or to subject them to the amount of pressure sufficient to make them thin enough for observation with high powers. Nevertheless, not a few observers still use water and solutions of which water is the principal ingredient. To this no objection can be made; but not content with working on in their own way, some of these individuals do all they can to



underrate the importance of observations made upon different principles. If anyone makes out new points of structure by any new method, all that such an authority who differs has to do is to state that he has not been able to see the structure described by so and so. *Authority* too often denies the existence of what it has itself been unable to see. Many authorities deny the existence of what they have not seen, while they have not taken the pains to try the only method of demonstration by which the appearance in question could be seen; or, without having ever seen points of structure described by others, and without denying the truth of their observations, they say such an arrangement does not exist in the corresponding tissue of some animals closely allied to the one in question.

Everyone now aiming at original observation upon the minute structure of living beings, must become skilled in the use of far higher magnifying powers than those generally employed. In the preceding Chapter I have fully discussed the principles which should be borne in mind in the preparation of the specimens. For all success depends upon this, and the observer should certainly begin with the use of low powers. As he improves in the mode of making specimens, he may advance to the use of the higher and the highest powers. My own opinion is, that an entirely new field is opening out for exploration, and that a vast number of new anatomical facts will consequently be discovered during the next few years, by the aid of new methods of investigation. Many of the points yet remaining for investigation, involve questions of fundamental importance; and when these are determined, a great change for the better will be observed in physiology. It seems to me that minute anatomy has been far too little studied. We ought to have a thorough knowledge of mere structure before we begin to discuss action; but it is too often the case that mere speculations are received, and widely taught, although anatomical facts demonstrate them to be unsound.

Much has been said, and doubtless yet remains to be said, against the use of high powers. But, even if it be admitted (but I do not myself admit this) that nothing more can be seen by the use of a very high power than by one that magnifies much less, there cannot be the slightest doubt about the fact, that objects which would pass quite *unnoticed* by the latter, must at once *attract attention* if examined by the former. If high powers were

of service only in bringing important but most delicate peculiarities of objects under observation—if by their use the attention were merely directed to minute points which would otherwise pass unobserved, it would be necessary to employ them in carrying out advanced work.

There are some branches of microscopical inquiry in which very high magnifying powers are absolutely necessary. For example, in such investigations as those which have lately been carried on by M. Pouchet and M. Pasteur, many of the more minute organisms can only be seen by a power magnifying upwards of 1,000 diameters. Bacteria, magnified 1,800 and 3,000 diameters, are represented in Plate LI, Figs. 247, 248, 249. If still higher powers had been brought to bear upon the specimen, organisms still more minute than any represented in these figures would probably have been demonstrated. The most minute of such living organisms discoverable by a power of 10,000 linear, has been living and growing for some time before it attained sufficient dimensions and density to be visible to us. I believe if magnifying power could be efficiently increased to ten times ten thousand diameters, we should still only be able to see one particle of living matter increasing in size, and giving rise to new particles, which in their turn become detached—and so on. We should see nothing like the aggregation of particles, or the coalescence of already existing particles, of inanimate matter to form a mass of living matter. We should see, I believe, nothing but the growth and division of living particles already in existence. We might, however, be able to demonstrate germs of a degree of minuteness not yet thought of. But there is another matter of the greatest importance in the consideration of this most difficult question, which has almost entirely escaped notice. Besides extreme minuteness in mere size, extreme tenuity or transparency may interfere with the definition of an object. Now, the greatest difference is observed in object-glasses in this particular. The best object-glasses will define clearly and accurately, bodies, which, from their transparency, are quite invisible under objectives only slightly inferior to the first. I feel quite sure that many statements recently made with reference to the mode of formation of the lowest forms of life, by the process of aggregation of particles, arise from imperfect means of observation, and that the real germs existed before they possessed sufficient density to be recognised by the object-glasses employed.

The following circles represent the size which objects would appear in the microscope under the respective magnifying powers as stated :—

Molecule  $\frac{1}{10000}$  of an inch in diameter  
× 250 linear.



Molecule  $\frac{1}{10000}$  of an inch in diameter  
× 250 linear.



The same × 700.



Molecule  $\frac{1}{10000}$  of an inch in diameter  
× 700.



The same × 1800.



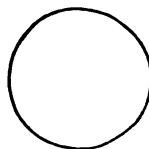
The same × 1800.



The same × 4000.



The same × 3000.




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$\frac{1}{1000}$  of an English inch, magnified 250 linear.

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$\frac{1}{1000}$  of an English inch, magnified 700 linear.

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$\frac{1}{1000}$  of an English inch, magnified 3000 linear.

**305. Of the Highest Magnifying Powers.**—Messrs. Powell and Lealand succeeded in producing a sixteenth, magnifying a thousand diameters as long ago as the year 1840. In 1859 I was engaged in studying the arrangement of the nerves in voluntary muscle, and succeeded in preparing, by the process given in § 300, some exceedingly thin sections, in which most delicate nerve fibres could be distinguished, but these were very pale and transparent, and the appearance was such as to lead me to the inference that in many cases apparently single fibres really consisted of several very fine fibres. I desired, therefore, to examine the specimens with a much higher power, and I begged



Messrs. Powell and Lealand to endeavour to make for me a glass with a magnifying power double that of the sixteenth. In the year 1860, I received from these makers the first twenty-sixth ever made, which magnified 1,800 diameters. Of this glass I have now had great experience, and can speak of it as a most excellent working glass. That it defines exceedingly well, and admits plenty of light, is obvious from the fact that it will allow of the tube of the microscope being increased considerably in length. By a *working glass*, I mean one that can be employed without great trouble or difficulty, and does not require any elaborate arrangements with regard to illumination, adjustment, &c. In fact, it works well even without a condenser of any kind, the common concave mirror being alone used. There is plenty of room for focussing, although, of course, specially thin glass or mica must be employed. I have made and published many drawings of tissues of the higher animals magnified with this glass, and it need scarcely be said that, as it can be brought to bear upon textures of this class (even bone and teeth), thin sections of which are obtained only with great difficulty, it must be readily applicable to other departments of microscopical inquiry. Object-glasses of very high magnifying power have been made by other makers, but those who have compared them with Powell and Lealand's twenty-fifth consider them inferior to this glass. Hartnack, of Paris, has modified the correction of the lenses, so that a thin stratum of water between the glass cover of the object and the last lens of the object-glass is required to make the definition perfect. I have not been able to convince myself of the superiority of this plan over the ordinary method, although I have not had sufficient experience to enable me to express a positive opinion upon the matter. An objective of high magnifying power (a twentieth) has lately been made by Messrs. Smith and Beek. I have had an opportunity of comparing this glass with the twenty-fifth of Powell and Lealand. The magnifying power is about one-third less, and it appeared to me that the definition was not so good. The amount of light admitted was ample. It is, however, exceedingly difficult to express in words the merits of one glass as compared with another, and there can be no doubt that an observer who has used one glass very much, especially if he has made new observations by its aid, is almost of necessity prejudiced in its favour; and I confess that, unless I had worked with a glass for a considerable period of time, I would



not express a decided opinion as to its qualities. The difference between the working powers of the glasses of the best makers is, at most, very slight, and not to be demonstrated without the most exact and careful examination. At the same time, it is certain that the slightest advantage in defining power ought not to be underrated, for it may enable the observer to see some scarcely perceptible, but nevertheless most important, points not observed before, and in some instances the very slightest advantage of this kind may necessitate a complete alteration in general views up to that time received as true, and considered even to be fixed and unalterable. Improvement in the means of observation is of the utmost importance, and, however slight, always leads to the discovery of new facts.]

**306. Of the Covering Glass.**—The cover may be made of a very thin plate of mica, but glass possesses several advantages. Messrs. Chance, of Birmingham, have lately succeeded in manufacturing in quantity glass sufficiently thin for the  $\frac{1}{4}$ th. This is supplied by Messrs. Powell and Lealand. For mere examination of specimens, thin plates of mica answer well, and they may even be used for mounting the preparation permanently; but as it is difficult to clean the surface without scratching it, it will be found better to use thin glass as the covers of specimens which are to be kept permanently.

**307. Illumination of Objects Magnified by very High Powers.**—Successful observation with very high powers is mainly dependant upon illumination. Indeed, by ordinary means it is not possible to obtain a light sufficiently intense to illustrate an object magnified 3,000 diameters. I have tried with greater or less success many different plans, and have used prisms, concave mirrors, and various kinds of condensers. I have, however, arrived at the conclusion, that the most satisfactory results by far are obtained by the use of Kelner's eye-piece as a condenser, as suggested by my friend Mr. Brooke (*see* page 24). By this means I can obtain a light sufficient for a magnifying power of 10,000 linear. I have tried the lime light, but have not found that it possesses any advantages over the belmontine or paraffin lamp, while the glare from it is much greater.

**308. Method of Increasing the size of the Image without**

**altering the Object-Glass.**—Supposing the limits of magnifying power of the object-glass to have been reached, there are yet methods by which the dimensions of the image may be greatly increased. The eye-piece may be changed for a deeper one, or the distance between the object-glass and eye-piece may be increased. In practice, I have found that the latter plan is so much the most advantageous that I now never use a deep eye-piece.

The  $\frac{1}{2}$  objective being applied when the tube is increased in length, so that from the lowest glass of the object-glass to the eye-glass of eye-piece, the distance measures—24 inches, the magnifying power corresponds to upwards of 10,000 diameters. 20 inches about 6,000. 15 inches about 2,600. 11 inches about 1,800. When the tube is thus increased in length, there is often some reflection from its interior which renders the image indistinct. This inconvenience may be remedied either by increasing the diameter of the microscope tube to about  $2\frac{1}{2}$  inches, or by lining the ordinary tube with black velvet.

Of course, the practical utility of increasing the magnifying power entirely depends upon the character of the specimen. The preparation of specimens has already been considered, and it has been shown that the preparations to be examined by very high powers must be immersed in the strongest glycerine that can be procured.

In delineating the appearances observed, I never represent a structure more highly magnified than is necessary to bring out the points; but I find that as I improve my method of preparation I desire higher magnifying powers, and I am quite certain that great advantage will be reaped when powers far higher than any yet made, or thought of, shall be brought to bear upon many structures. The question of preparation is scarcely more than a mechanical one, and new and more exact means of preparation will soon follow improvements in the optical part of the microscope.

### 309. Of Drawing Objects magnified with very High Powers.

—It is extremely difficult to use the neutral-tint glass reflector with the highest powers, and the slightest vibration of the instrument causes confusion in the lines, so that, in practice, I have found the best method is, to measure the distance of the several parts of the object with compasses, and then, having fixed these

represented in Plate L, Fig. 230x. Now, such particles cannot be termed cells, according to the ordinary definition of that word. Yet each consists of germinal matter, with probably a thin layer of formed material upon its surface. Each of these may increase in size by the absorption of nutrient pabulum into its substance, and may then divide and subdivide into separate portions. The mucus corpuscle, represented in Plate LII, Fig. 241, also consists of a mass of germinal matter which, as it lies in the mucus or formed material, exhibits movements as shown by the dotted lines. The white blood corpuscle, Plate L, Fig. 236g, is another example of germinal or living matter, which is invariably colourless, and which, as is well known, exhibits slow movements. These movements I believe to be *vital movements*.\*

The different changes which occur in the germinal matter and result in the production of tissue and of various substances exhibiting no definite structure, will be readily understood by reference to Plates L, LI, LII, LIII, LIV, LV.

The character of germinal or living matter can also be studied very readily in the common *Amœba*. These low forms of living beings are generally found in great numbers in water containing a little decomposing vegetable matter. If carefully examined under the  $\frac{1}{2}$  of an inch object-glass, the amœba will be observed to alter in form. At various parts of the circumference protrusions will be observed. These protrusions consist of the material which forms the basis of the amœba. It will be observed that this moving material is perfectly transparent, and in it no appearances of structure can be discerned. It is true that granules and foreign particles may be seen embedded in it, but the matter in which the motor power resides is perfectly clear and transparent. Motion is *communicated* to the solid particles by the movement or the transparent living matter.

In the case of blood corpuscles (Plate L), it appears that the outer part of the germinal matter becomes resolved into the viscid red coloured matter characteristic of the red blood corpuscle. The manner in which this takes place will be readily understood by Figs. 235, 236, and 237 in Plate L. In mammalia, it seems that the whole of the germinal matter of the (white) blood corpuscle is soon resolved into red colouring matter. Thus the red blood corpuscle results. The latter is not *living*, but consists

\* See a paper by me "On 'Contractility' as distinguished from purely vital movements."—*Mic. Journal*, July 1864.



of matter in a colloid state, which very soon passes into a crystalline form. In some instances, as in the case of the blood corpuscle of the Guinea pig, this change occurs within a very short time after the corpuscle has ceased to move, as when it is withdrawn from the circulation of the animal and placed upon a glass slide. In Figs. 238, 239, 240, some of these crystals formed from the red corpuscle of Guinea pig's blood are represented.

Another simple case, showing the formation of formed material from germinal matter, may be studied in cuticle, or in the cells upon the papillæ of the tongue. At first there is but a very thin layer of formed material upon the surface of the germinal matter, and this is soft, so that the mass may divide, and each portion may be invested with a thin layer of this soft formed material. Nutrient pabulum passes through it to the germinal matter within, and a portion of the latter undergoes conversion into formed material. The germinal matter increases, while at the same time new formed material is produced. This is shown in Fig. 242, *a*, *b*, *c*, and *d*; but now (*c* and *d*) a thick layer of formed material has resulted, which only permits a very little pabulum to pass through. The entire cell does not, therefore, increase in size; but the conversion of germinal matter into formed material still proceeds, so that at last but little of the latter remains, as is represented in *e*.

But let us consider the wonderful effects which ensue from a change of the circumstances under which the cell is placed. Suppose the hard and formed material which interferes with the access of pabulum to the germinal matter to be ruptured, or softened by the action of fluids, so that pabulum may more readily come into contact with the germinal matter. What happens? Why, the latter increases. It absorbs this nutrient matter, and may even take up the softened and altered formed matter, which was itself produced from germinal matter at an earlier period. These stages are seen in Fig. 244, *f*, *g*, *h*. In Fig. 245 *i*, the original mass has divided into several, and in *k* these are set free, and being now freely supplied with pabulum, they grow and multiply rapidly. Such are the changes which are considered to result from what is called "Irritation," and which constitute the essential phenomena of "inflammation." *Irritation*, I have tried to show, really means but this,—that the access of pabulum to germinal matter is facilitated, and the protective external covering of formed material is removed or



absorbed pabulum. Oxygen acts upon the lifeless matter of the cell rather than upon that which lives. It does not support life directly, but is necessary to the continuance of life, because it alone may be instrumental in converting the products of decay and death into soluble substances which can be readily removed.

In the cell phenomena, above described, the 'nucleus' takes no part. What, then, is the 'nucleus,' of which many examples will be seen in the drawings in the plates. The nucleus also consists of germinal matter. It may be regarded as a new centre arising in a pre-existing centre. In many masses of germinal matter there are, in fact, two or three series of centres, one within the other. The nucleus is also germinal or living matter, and it has appeared as a new centre within germinal matter already existing. The vital power or force, whatever its nature may be, always manifests itself in a direction from centres,—that is, living particles of matter move invariably in this direction, and as they move farther and farther away from the centre, their *vital power* becomes less. Living particles do not aggregate together to form one mass, but one mass may divide and separate into a vast number of distinct particles.

The term 'cell' was considered to be applicable to all the elementary parts of which organic bodies were composed, and if it had not been laid down arbitrarily that a 'cell' involved the existence of a 'wall,' certain 'contents,' and a 'nucleus,' there would be far less difference of opinion among observers than now exists. Distinct properties are still attributed to these parts respectively, although no one has ever been able to show that they really possessed the offices assigned to them. It is obvious, however, that a small particle of living matter will not fall under the definition given of a cell, nor is it possible, by any reasonable interpretation of the terms employed, to bring white blood corpuscles and a host of other objects into the cell category. To include these the definition must be totally changed. The difficulty of including many bodies under the old definition, combined with an implicit faith in its truth, has led many observers to affirm the existence of a cell-wall, although none was present, and at last the supporters of the old cell doctrine have taken refuge in the idea that the 'cell-wall' may itself be fluid and capable of running together like the film of a soap bubble. The moving matter of the white blood corpuscles, the granular matter around the co-called nuclei of muscle, the col-

tents (in parts, or entire) of the vegetable cell, have been called 'protoplasm,' but those who have employed this word have not accurately defined what they include under it, and they still consider the nucleus as a distinct object performing special functions.

To avoid entering into a long and tedious discussion upon the meaning which should now be assigned to the words in general use, I have employed new terms to explain the conclusions I have arrived at in endeavouring to ascertain the actual meaning of all the different structures met with in various tissues. These new terms are those employed in the foregoing account, namely: 1. *Germinal or living matter*; and 2. *The formed material*.—I apply the term germinal matter only to that which lives, changes, converts, germinates, &c., while the formed matter never possesses any of these properties. Pabulum may become germinal matter, the latter formed material—cell-wall, or intercellular substance—and this last may be disintegrated. The really important point is, that formed material of every kind was once germinal matter, and that new matter is deposited in one definite direction only, namely, from within from a centre, so that the oldest part of the formed material is that which is most external.

Moreover, there exists very great confusion among writers with regard to the definition of the terms they employ in describing the structure of, and changes taking place in, cells, and no wonder, for the meaning of these terms undergoes great modification from year to year.

The living cell, then, consists of *germinal matter* and *formed matter*. The first is the matter upon which alone growth, formation, conversion, and multiplication depend, and these *vital* processes never occur unless germinal-matter, with its marvellous vital power, is present. The formed material owes its properties partly to the changes occurring in the matter when in the living state, partly to the external conditions present when the living matter was undergoing change, in fact, at the moment of death; so that I distinguish vital from the physical and chemical changes of living beings, and maintain that in all, matter exists in two states; the first being that in which the vital changes go on, while the last is the seat of chemical and physical alterations. That force or power which compels the matter to assume temporarily the peculiar state characteristic of all living matter, but of living matter alone, I call *vital power*. Of its real nature we know nothing; but although, in the present

state of knowledge, we can form no conception of the nature of this wonderful power, there are, it seems to me, very strong arguments against the notion, now very prevalent, that it is a kind of ordinary force, or that it corresponds to what we call the peculiar *property* of each different inorganic substance, by virtue of which each exhibits certain constant crystalline forms, certain constant behaviour towards other substances, &c.

From my observations, I can draw but one inference with regard to vital power, namely, that it is not any modification of any known ordinary force. It is not another mode of motion. It is only manifested under certain conditions, but it does not *result* from those conditions. That it does not correspond to the *properties* of ordinary inanimate bodies, is evident from the fact, that it is a power capable of being transferred from complex particle to particle, and not only controls the manifestation of ordinary forces, but gives rise to the formation of certain compounds and structures, which are only to come into use at some distant time. A fully formed organ is not first represented by a microscopic organ of precisely similar structure, but by a mass without structure at all, and the fully formed tissues are preceded by the production of several less elaborate structures. Hence this "vital power" governs not only the present changes which present matter is to undergo, but prepares in advance for changes which are to occur at a future time. It prepares, as it were, for the formation of structures long before the compounds are produced, from which those structures are to be made. While ordinary force seems for the most part to affect the surface of masses, vital power acts from the very centre of the most minute particle—new power seems, as it were, to be for ever emanating from the very centre of particles of matter already under the influence of this power. While ordinary force may change its form, it cannot cease or be annihilated; but there is no evidence to show that vital power changes its form, while, as far as is known, it may be said to cease,—since no one has yet proved that, when living matter dies, any kind of force is set free; and, although it has been asserted that more force is taken up in the formation of a brain cell of a man than in the formation of a vast quantity of vegetable tissue, there is no evidence in favour of such an hypothesis but the dictum of speculative writers.



As the explanation of the following plates is far too long to insert at the foot of each plate, I append it here.

EXPLANATION OF PLATES I TO LV, ILLUSTRATING THE  
STRUCTURE OF THE CELL.

PLATE L.

Fig. 230.—*a*. The smallest visible particles of germinal matter. *b*. Small collections of germinal matter, with a little formed material between them (as in mucus). In one, portions are seen to project, and if these were detached each one would grow and give rise to new masses. *c*. Germinal matter, with a very thin layer of formed material on its external outface (cell-wall). *d*. Same as the last, but with a new centre of growth (nucleus), now comparatively quiescent, but capable of assuming active growth, appearing in the germinal matter. If *c* were exposed to unfavourable conditions the whole would be destroyed, but under similar circumstances the nucleus of *d* might alone resist these influences, and the conditions becoming favourable, would grow and produce new elementary parts, although all but this small portion of the germinal matter had been destroyed. *e*. A thick layer of formed material, the whole of which was at one time in the state of germinal matter. *f*. Secondary deposits commencing to appear amongst the germinal matter, as fatty matter is precipitated amongst the germinal matter of the fat vesicle. *g*. A further stage of the same process. *h*. Separate masses of secondary deposits, as in the starch-holding vegetable cells. *i*. Deposition of formed material or secondary deposit in successive layers on the inner surface of the original capsule, spaces or intervals in which currents are continually setting in opposite directions during the life of the germinal matter being left. *k*. Germinal matter and formed material which is granular, the particles of which are becoming resolved into several substances as takes place in the elementary part of the liver (liver cells). *l*. Formation of fibres from germinal matter. *m*. Germinal matter belonging to and taking part in the formation of the walls of a tube.

Fig. 231.—Five young starch-holding cells of the potato, showing the outer thin layer of formed material (cell wall), the germinal matter (protoplasm, primordial utricle) with small starch globules precipitated amongst it, also the nucleus and nucleolus.



Fig. 232.—A fully formed starch-holding cell of the common potato. The primordial utricle has been removed from the right half, in order to show the starch globules within more distinctly. The nucleus and nucleolus are seen in the remaining portion. Around are portions of neighbouring cells, the wall of which are separated here and there by compressed bubbles of air, indicated by the very dark shading. Below are seen some elongated cells with nuclei and nucleoli. These probably become spiral vessels.

Fig. 233.—One of the large cells with thick walls, containing no starch, but exhibiting pores which are, however, closed externally by a thin layer of the original capsule (cell wall).

Fig. 234.—A piece of the wall of one of the cells figured in 233, magnified 700 diameters, showing the manner in which the material applied to the thickening of the wall is added, layer after layer, upon the inner surface. The innermost layers are to the left hand, in the position in which the figure is placed.

Fig. 235—*a*. Division of very young white blood corpuscles of the frog, and formation of the outer coloured portion. *b*. "Nucleus," nucleolus, and outer red formed material. *c*. Change in form of outer red formed material, showing that it consists of soft viscid matter. *d*. Movement of germinal matter towards the surface of the red formed material.

Fig. 236—*g*. A young corpuscle of the frog, not yet coloured. *h*. A young red corpuscle; formation of coloured portion. *i*. A young red corpuscle; a part of coloured portion fully formed. *j*. A young red corpuscle; coloured portion and nucleus.

Fig. 237—*k*. An oval corpuscle of the frog, become spherical in weak glycerine. *l*. Division of germinal matter of "nucleus;" in the viscid coloured matter of the corpuscle. *m* and *n*. Old red corpuscles which have not assumed the spherical form. Nearly the whole of the germinal matter has been converted into coloured matter.

Fig. 238.—Disintegration of the red blood corpuscles of the Guinea pig, resulting from the application of slight heat. Some have separated into several small particles, each of which has assumed the crystalline form.

#### PLATE LI.

Fig. 239.—Changes in form of red blood corpuscles of Guinea pig after removal from the body, without the application of heat, or the addition of any chemical reagent.

Fig. 230.



p. 224.

Fig. 231.



Fig. 233.



p. 237.

Fig. 234.



p. 227.

Fig. 235.



Fig. 232.



p. 227.

700.

Fig. 236.



$\times 1500.$



$\times 1800.$

p. 224.



$\times 1800.$



$\times 700.$

Fig. 237.



$\times 700.$



$\times 700.$

p. 224.



$\times 700.$




$\times 700.$

Fig. 238.



$\times 700.$

p. 225.

1000th of an inch   $\times 700.$

[To face page 232.]

11

11

Fig. 240.—Disintegration of the red blood corpuscles of the Guinea pig resulting from exposure to heat.

Fig. 241.—Mucus corpuscle. From the mucus of the throat, showing the different forms it assumed within a minute. The nuclei are seen in the centre of the parent mass. Portions of this have moved away some distance and two are detached. These would grow and form new mucus corpuscles. Nuclei might arise in the portions detached. The movements observed seem to be independent of the nucleus. The nature of these movements has not yet been explained, but I consider them to be "vital" movements.

Fig. 242.—Normal epithelial cells in different stages of growth. *a*, is a growing cell near the vascular surface; *b*, is an older cell; *c*, is more advanced; *d*, is still older; and *e*, is fully advanced, and about to be cast off. In all the drawings, the roundish granular mass represents the *living or germinal matter*, and the outer faintly-shaded layer the *formed material*, which was once germinal matter. In the normal state, nutrient pabulum gradually passes through the formed material into the living or germinal matter. Certain constituents of the pabulum immediately acquire the same wonderful powers as the germinal matter already existing, while the particles of germinal matter upon the surface of the mass undergo change, and are resolved into the formed material of the cell-wall, and into other matters which pass away. This formed material is always formed *from within*, so that the layers first produced are pushed outwards by the formation of new matter within. This may be incorporated with that which was first produced, or several successive layers may be formed one within the other. The germinal matter is the only *formative, living, growing, or active part of the cell*. Germinal matter forms germinal matter from pabulum, and becomes converted into formed material. The latter is inanimate, and cannot produce matter like itself.

Fig. 243.—An epithelial cell, with the germinal matter exposed, so that pabulum would come into contact with it freely. The outer formed material is torn or ruptured mechanically, as in a scratch or in consequence of a prick by an insect ('irritated').

Fig. 244.—Epithelial cells, the formed material of which is softened and rendered more permeable to the nutrient pabulum. Under these circumstances the mass of germinal matter increases in size, as in *f*, *g*, and soon begins to divide into smaller portions.



Parts seem to move away from the general mass (*h*). These at length become detached, and thus several separate masses of germinal matter, which are embedded in the softened and altered formed material, result (Fig. 245, *i*).

Fig. 245.—*i*, *k*. The germinal matter of epithelial cells increasing very rapidly as in 'inflammation' and giving rise to 'pus corpuscles.' When the formed material is ruptured or absorbed at one point, the germinal matter soon escapes, leaving the remains of the cell-wall (formed material of the original cell) behind. Or the masses of germinal matter increase in size, and even live at the expense of the softened formed material, which was formed from the original mass of germinal matter, and at length escape (*k*). The free masses now in contact with the pabulum grow and multiply rapidly. Each forming upon its surface a more or less viscid material, which corresponds to the formed material of the cell, but is softer and much more readily permeated by nutrient matter.

In this way the so-called inflammatory product *pus* results, and the abnormal pus-corpuscle is produced from the *germinal or living matter of a normal epithelial cell, in consequence of the germinal matter of this cell being supplied with pabulum much more freely than in the normal state.*

I have arrived at these conclusions from studying the changes which actually occur in specimens coloured with carmine, by which the "*germinal matter*" can in every case be readily distinguished from the "*formed material*." The nature of the changes occurring in cells in inflammation can easily be explained if the artificial nomenclature of cell-wall, cell-contents, nucleus, be given up. In all acute internal inflammations a much larger quantity of inanimate pabulum is taken up by certain cells and converted into living matter than in the normal state. Hence there is increase in bulk. Cells of particular organs, which live but slowly in health, live very fast in certain forms of disease. More pabulum reaches them, and they grow more rapidly in consequence.

Fig. 246.—Vibratile filaments and minute particles consisting of viscid coloured matter. Blood corpuscles of human subject after being subjected to heat,  $\times 1800$ . These filaments very closely resemble bacteria, and must not be mistaken for them.

Fig. 247.—Bacteria or vibriones magnified 1800 linear. From the mouth.

Figs. 248, 249.—Vibriones and fungi in old epithelial cells of the mouth, magnified 3,000 linear.

PLATE LI.

Fig. 239.



Fig. 240.



Fig. 241.



Fig. 242.



Fig. 243.



Fig. 244.



Fig. 245.



Fig. 246.



Fig. 247.

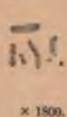


Fig. 248.

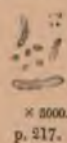


Fig. 249.



1000th of an inch 1 ————— x 1800.



## PLATE LII.

Fig. 250.—Germination and growth of common mildew stained with carmine,  $\times 1800$ . *a.* Spores, slightly swollen, showing *formed material* outside and *germinal matter* within. *b.* Spherical particles of germinal matter set free from a spore. *b.*  $\times$  Spore, with its external envelope of *formed material* opened. *c.* Spherical particles of germinal matter within spore increasing in size and dividing. Pores visible in envelope. *d.* Some of the particles of germinal matter have increased at one point, and have extended from the spore. They continue to grow rapidly, being protected only with a thin layer of *formed material*. *e.* A spore which has much increased in size, but from which no off-shoot has proceeded. The thick successive layers of *formed material* are seen. The oldest layer is outside, the youngest in immediate contact with the germinal matter. *f.* A spore from which an off-shoot has proceeded. The germinal matter of the off-shoot has been destroyed, while that of the spore retains its vitality. *g.* A part of the thallus, the germinal matter of which is dead. The external tubular membrane *retains its physical characters*. *h.* Portions of thallus in which the germinal matter is increasing most rapidly in certain spots. Here branches are formed. *i.* A young branch. *k.* An older part of the thallus, showing the septa of *formed material* and the points at which the germinal matter was originally continuous. The *formed material* is thicker than in *h* or *i*, and the germinal matter is a little shrunk within its tube. *l.* A spore from which an off-shoot has proceeded. The envelope is seen to be very thin at the summit, where growth is occurring most rapidly. *m.* Spore with two off-shoots from opposite surfaces. These have grown and are giving off branches, but the continuity between the germinal matter in these and that in the spore still exists. *n.* The germinal matter of the spore is dead, and forms a collapsed mass within, while that in the off-shoot retains its vitality and is increasing. *o.* A very old spore which has germinated and grown much larger. An off-shoot has also proceeded from it, but, from being exposed to conditions unfavourable to its free extension, the *formed material* has increased enormously in thickness by the deposition of successive layers on the internal surface. *p.* A part of one of the stems which grow



into the air, bearing on its summit oval capsules, from the germinal matter of which the spores are formed. *r.* A separate capsule.

Fig. 251.—Two portions of the stem of a sea-weed. *a.* The summit of a growing shoot. Vegetable organisms grew upon every part of the outer layer of the formed material. This is increased in thickness by the deposition of new layers from the germinal matter. The continuity between the masses of germinal matter is still seen. *b.* From an older part of the stem, showing the mode of formation of the spores.

These figures illustrate the following points:—

1. That every living organism, and every elementary part of an organism, consists of matter in two states. *Germinal matter*, growing, active, undergoing change. *Formed material*, which has been formed from the germinal matter, passive, and not capable of growing or of selecting nutrient substances.

2. That the *formed material* is on the outside of the germinal matter, and is increased in thickness by the deposition of new matter on its *inner* surface. The *outer* part of the formed material is the oldest; the inner that which has only just passed from the state of germinal matter.

3. That the masses of germinal matter are composed of smaller spherical particles, and these, again, of smaller spherules (Fig. 250, *b*).

Fig. 252.—One of the ganglion cells, with nerve fibres connected with it, from a nerve distributed to the pericardium of the ox. The large nucleus of the ganglion cell is seen, but in its substance, principally near the surface, a number of small oval nuclei, resembling those in the nerves, are also visible.

#### PLATE LIII.

*Distribution of finest nucleated nerve fibres to the very narrow elementary muscular fibres of the mylo-hyoid of the little green tree-frog (Hyla arborea) magnified 1,700 diameters. Drawn on the block by the author.*

The elementary muscular fibres are marked *g*, *h*, *i*, *k*. *k* is a very young one, slightly stretched; *i* is a fully-formed muscular fibre; *h*, another stretched in its central part. The nuclei of these fibres exhibit some difference in size and form. Nucleoli are distinct in all, and in the fibre marked *g* the nuclei, which were coloured by carmine, exhibit three different intensities of

Fig. 250.



p. 226.

x 1500.

Fig. 261.



p. 226.

x 350.

- Fig. 262.



x 700.

1000th of an inch

1500.

[To face page 250.]



colour,—the dark central spot, “nucleolus,” being most intensely coloured, as indicated by the shading in the drawing.

*a* is a nerve-fibre which was followed over more than twenty elementary muscular fibres from a dark-bordered fibre. One of the subdivisions of this fibre is seen at *f*, where it again runs with a very fine dark-bordered fibre (*o*). The dark-bordered fibre (*o*) was some distance higher up in the specimen, but its place has been altered in order to avoid the necessity for a still larger drawing. Above *b*, a nucleus of a very fine nerve fibre is seen. Such nuclei lie upon the surface of the muscular fibres, external to the sarcolemma. The nucleus often *appears* as if it were within the sarcolemma (*c*), but the fibres proceeding from each extremity render such a position impossible. The relation of these nerve nuclei to the sarcolemma is seen at *l* in profile. The nuclei, as well as the fibres for a certain distance, often adhere to the sarcolemma very firmly: but in the thin mylo-hyoid muscle the course of the fibres over or under, but always *external* to the muscular fibres, may be readily traced if the muscular fibres be separated slightly from one another, as represented in the drawing.

At *d* fine nerve fibres accompanying the fine fibre continued from the dark-bordered fibre, as described in the “*Philosophical Transactions*” for 1862, are represented. Such fibres are also seen at *e* and *f*.

*m*, *n*, and *o* dark-bordered fibres, with nuclei, near their distribution. *m* would probably pass over sixty or 70 muscular fibres, and *n* over perhaps twenty, before it divided into fibres as fine as those seen at *b*, *e*, *f*, *l*.

*p* a very fine capillary vessel with a nerve fibre running close to it.

*q* a bundle composed of six very fine nerve fibres near their distribution. These fibres exhibit a very distinctly beaded appearance, which is also observed in many other fine fibres in different parts of the specimen.

Traces of connective tissue are seen in all parts near the fine nerve fibres and around the muscular fibres. Here and there some very fine connective tissue fibres, which were not altered by acetic acid, are represented. These represent the remains of fine nerve fibres, which existed in a state of functional activity at an earlier period.

The drawing, with the exception of the position of the nerve



## HOW TO WORK

above-mentioned, is an actual copy from nature. The position of the muscular fibres, the form and general characters of the so-called nuclei, and the position and size of the nerve fibres and their nuclei, have been carefully preserved.

I have traced the very fine nerve fibres in so many instances from one trunk to another ramifying at a very considerable distance, that I cannot believe any true terminations or ends

## PLATE LIV.

Fig. 254.—One of the ganglion cells embedded in the trunk of a nerve near the lumbar nerves of the green tree-frog (*Hyla arborea*). The cell was isolated by dissection and pressure in glycerine. A *straight fibre* is seen to be continuous with the central part of the cell, and a *spiral fibre or fibres* with its circumference. The matter of which the body of the cell is composed passes into the fibres. The germinal matter was coloured with carmine. The broad fibre forming the continuation of the nucleated spiral fibre is a true '*dark-bordered fibre*.'  $\times 1,800$ . Jan., 1863. It is observed in the specimen that the straight fibre passes in one direction in the trunk of the nerve, while the fibre continuous with the spiral fibre passes in the opposite. This is one of the anatomical facts which has led me to conclude that nerve fibres always form complete circuits.

## PLATE LV.

Fig. 255.—A mass of imperfectly developed ganglion cells. *a*, a mass dividing into two. *b*, connective tissue corpuscle. *c*, nerve distributed to capillary vessel, *e*. *d*, a more advanced ganglion cell exhibiting straight and spiral fibres; its straight fibre is much thicker than any in the bundle of fine fibres passing into the body of the ganglion.  $\times 1,800$ . Every one of the cells represented in this figure would at length grow into a distinct cell, like that seen in Plate LIV.

Fig. 553.



Distribution of finest nucleated-Nerve Fibres to the Elementary Muscular Fibres of the Mylo-hyoid Muscle of the little Green Tree Frog (*Hyla Arborea*). Drawn on the block by the Author, from a specimen magnified 1700 diameters (the first twenty-fifth made by Messrs. Powell and Lealand). The diameter of each muscular fibre corresponds to that of a human red blood-corpuscle.

SCALE,  $\frac{1}{16000}$  of an English inch  $\times$  1700 diameters.

16000

[To face page 238.]



Fig. 254.



1000th of an inch



x 1800.

[To follow Plate LIII.]






1

Fig. 250



1000th of an inch   $\times 1800$ .

To follow Plate LIV.



## CHAPTER XII.

OF MAKING AND RECORDING MICROSCOPICAL OBSERVATIONS.—*Of drawing Inferences from Observations. FALLACIES TO BE GUARDED AGAINST IN MICROSCOPICAL INVESTIGATION.—Errors of Observation—Of the Commencement and Termination of Tubes—On the Difficulty of seeing Structures from their extreme Transparency—Fibres and Membranes produced Artificially by the Action of Reagents—A Fibrous Appearance produced in Structureless Membranes—Collections of Oil-globules appearing as if within a Cell—On the accidental Presence of extraneous Substances.* OF RECORDING MICROSCOPICAL OBSERVATIONS.—*Exactness of Description—Of the Importance of making Sketches.*

310. *Of making Observations upon Specimens in the Microscope.*—The eye of the observer requires much careful education, before he is able to appreciate fully the character of the structure which he is examining. If, upon examination, a specimen does not appear to him to justify the description or delineation which some observer has given of a similar structure, he must not too hastily infer that the author has been recording the results of his imagination rather than observed facts. We must remember that the conclusions which have been arrived at are probably the result of a very long and patient investigation, deduced from examining a specimen under very different circumstances, after the application, perhaps, of various chemical reagents, and after ascertaining the effect of different refractive media. From the remarks made in Chapters V and VI, some idea may be formed of the many different operations which are necessary to demonstrate conclusively the anatomy of a single tissue. The observer must not, therefore, be too hasty in deciding upon the nature of an object in the microscope; neither must he infer that what he has not been able to see does not therefore exist.

Some fall into an error of the very opposite description, but



not less detrimental to forming habits of correct observation. Led away by their imagination, they think they see everything which has been delineated, or which they have heard described; the observations of authors are confirmed in expressions closely resembling the original, and thus, in point of fact, their own testimony is brought forward, though not directly by themselves, a second time in favour of their original doctrines, without any real confirmation of the accuracy of their views being advanced. In this manner errors have been propagated and increased to an extent almost incredible, and years of laborious investigation have been spent in overthrowing statements which had never resulted from actual observation, in the first instance. Sometimes an idea, taking for its ingenuity and novelty, but having no foundation in fact, is seized by a number of persons, and supported by so many different observations, that it comes to be received as true, and is perhaps believed in for years, until some one reinvestigates the whole question, and demonstrates the absurdity of the doctrine.

**311. Of Drawing Inferences from Observations.**—No one engaged in the pursuit of any branch of natural science is more tempted to be led into too hasty generalisation than the microscopical observer. It is his duty, therefore, to avoid drawing inferences until he has accumulated a vast number of facts to support the conclusions at which he has arrived. True generalisations and correct inferences promote the rapid advancement of scientific knowledge, for each new inference clearly forms the starting point of a fresh line of investigation; but we must remember that, on the other hand, every false statement, regarded as an observed fact, forms a terrible barrier to onward progress, since, before the slightest useful advance can be made it is necessary to retrace our steps, it may be for a considerable distance, before we can hope to recommence our onward course. Again, a much greater amount of evidence is always required to overthrow a false conclusion than is sufficient to propagate the error; and there can be no task more unsatisfactory than to be compelled to subvert the opinions and deductions of others.

In this sort of inquiry I think it is a good plan *not* to make too minute notes during the progress of the investigation, but to retain, as far as may be, the facts observed, in the memory; and when the whole matter is made out, but not before, to begin

writing and recording the observations. Otherwise, imperfectly observed facts are liable to be set down as actual facts, and argued upon as truths; and thus the observer is perhaps gradually led more and more astray, until he ends by a conclusion utterly at variance with the real truth.

Scientific inquiry ought continually to advance, and we should be able to extend our researches from the point where they have been left by our predecessors, adding successively to what they had discovered; but the observations which we owe to them should not require correction. In not a few instances must we feel the highest respect for the careful observations of the older observers, and I fear it must be reluctantly confessed, that many of our modern researches are not carried out with the same patience, painstaking industry, and conscientious care as theirs, and for this reason are likely to be but short lived.

Now, there are many mistakes which an observer is very likely to commit, unless he be warned of their nature in the first instance. Some of these it will be well for me to advert to as briefly as possible.

#### FALLACIES TO BE GUARDED AGAINST IN MICROSCOPICAL INVESTIGATION.

Many mistakes have arisen in consequence of sufficient care not having been taken to prevent the introduction of various substances by accident. The most scrupulous cleanliness must always be observed in microscopical examination, and any foreign particles which may have accidentally come into contact with the preparation must be carefully removed before it is mounted. The plan of proceeding will depend much upon the nature of the texture and that of the foreign matter. Mere dusting with a camel's-hair brush, washing in a stream of water, or picking out the object with needles, are simple plans which are often efficient in a general way, but in some cases other processes are required. See §§ 86, 180.

**312. Errors of Observation.**—Every observer must be careful to avoid making erroneous observations. One is liable, not only to draw false conclusions from observations, but the observations themselves are not unfrequently erroneous. I propose to draw

attention to a few of what appear to me frequent sources of difficulty and doubt even to the most experienced.

**313. Of the Commencement and Termination of Tubes.**—The modes of commencement or termination of certain vessels or tubes have long been sources of dispute among observers. There are not a few instances where positive statements have been made that certain tubes commenced by coecal or blind extremities; while contradictions equally positive have been advanced by others, who have affirmed that the very same tubes commenced as a network, and presented no blind extremities whatever. It would be supposed by many that this point might be determined beyond all doubt by injecting the tubes with some coloured material. But this is not so. Injection will frequently run up to a particular point in the minute vessels, while no force which could be applied could drive it further onwards. Here, therefore, it accumulates, and often to a very considerable extent; the portion of the tube above the constriction being considerably dilated by the pressure which has been applied. Under these circumstances it is impossible to trace the further continuity of the vessel, owing to the extreme transparency and delicate nature of the tissue of which its walls are composed. Indeed, these may be quite invisible in an unprepared specimen. The observer is thus led into the error of supposing that such tubes terminate in blind extremities, whereas they may really form a network with large meshes, or they may be continuous with other structures beyond; and that which was taken for the termination or commencement of the tube may really be nothing more than a bulging in a central part of its course. In many thin sections of the kidney an appearance as if the tubes terminated in free blind extremities is produced in consequence of the convoluted portions of a tube lying in such a position that the recurved portion is immediately beneath the most superficial part of the tube. From a mere examination of the specimen it would be impossible for any one to say that this was not the case. In such instances the real disposition of the parts is only to be made out by a careful examination of the structure under different circumstances and prepared in various ways. Thus the idea that the tubes end by blind extremities may be shown to be quite inconsistent with the appearances observed in one particular mode of examining the texture. I am unable, however, to devote



much time to the consideration of this part of my subject, or I might review the various methods in which a tissue is examined, and show how by a consideration and comparison of the different facts observed, one is enabled at length to embody the results arrived at in several different inquiries, and form an idea of the real structure of the part. The remarks offered in Chapters V and VI may be referred to.

**314. On the Difficulty of Seeing Structures from their Transparency.**—Another fallacy arises from the great transparency of certain structures. Oftentimes a membrane may appear perfectly clear and transparent when in reality it is covered with a delicate layer of epithelium, which only becomes visible by being immersed in some special fluid or treated with some particular chemical reagent. On the other hand, there are instances in which an appearance resembling that produced by the presence of a cellular investment is perceived where no cells whatever exist. A peculiar corrugated state of uninjected capillaries, and the cells in the walls of the capillary vessels themselves, sometimes give rise to these mistakes. Basement membrane, from its extreme delicacy and transparency, is often only recognized by the folds into which it is thrown, or by the debris and granular matter which is accidentally adherent to it. Sometimes it becomes visible when immersed in a slightly-coloured solution, instead of in perfectly pure water.

**315. Fibres and Membranes Produced by the Action of Reagents Artificially.**—On the other hand, by the action of reagents a fibrous appearance is sometimes produced which, without care, may be mistaken for actual structure. The addition of acetic acid to many preparations frequently produces a swelling of the tissue, with the elevation of a clear membranous structure, which might be termed basement membrane, but which has really been formed in this manner. Thus the outer uncalcified portion of the cells of the enamel of a young tooth, may be made to swell up into a transparent mass, which was mistaken by Prof. Huxley for the membrana preformativa.

**316. A Fibrous Appearance Produced in Structureless Membranes.**—Clear, transparent, and apparently structureless membranes, when pressed, torn, and twisted, have a fibrous



appearance, and delicate vessels, whose coats are perfectly transparent when pressed and collapsed, may be very easily mistaken for a form of fibrous tissue. Both capillaries and fine nerve fibres may be mistaken for fibres of elastic tissue. Capillaries uninjected and stretched, can only be distinguished from fine nerve fibres with the utmost difficulty. If any doubt exist in such a case, it may always be cleared up by injecting the capillaries of the part with a clear transparent material, like plain size, when, if the fibrous appearance is not real it will be lost; while if fibres really existed, they would still be visible. The presence of capillary vessels in a structure has been entirely overlooked in consequence of their being collapsed and shrunken, in which state they have been described as fibrous tissue.

**317. Collection of Oil Globules Appearing as if within a Cell.**—Oil globules in fluid not uncommonly form small and nearly spherical masses or collections, which become covered with a certain quantity of mucus or viscid matter and granules, originally contained in the fluid, so that the little intervals between the minute oil globules become filled up; the outline of the mass is perfectly clear, and sharp, and well defined, and from mere ocular examination it is impossible to say that the oil globules are not enclosed in a cell-wall. A consideration of the circumstances under which such structures have been met with, will often assist us materially in determining their real nature. Such cells may be prepared artificially without the least difficulty, and in some cases it would not be possible to distinguish the artificially formed cell from the natural cell by mere microscopical examination in water, and the process of tinting would only act in cases of natural cells which were quite fresh. It need scarcely be said, however, that with respect to the formation of these bodies there is no analogy whatever. \* Of the artificial cell the most external part was *last formed*. It was deposited around a collection of particles. But in the natural cell the outer part is the *oldest part*. It was produced *before* the matter in the central part of the cell was formed. Probably the only observer who maintains that living cells are formed by the aggregation of granules, is Dr. Hughes Bennett, of Edinburgh, who thinks that a bacterium is formed by the coalescence of already existing particles. Dr. Bennett admits, however, that such simple

organisms multiply by division. See a controversy upon this subject in the "*British Medical Journal*," Jan., Feb., March, 1864.

**318. On the Accidental Presence of Extraneous Substances.**

—I believe, however, that the most common errors may be traced to the accidental presence of substances which are not familiar to the observer, and which are mistaken by him for bodies derived from the organisms he is investigating.

When we consider how minute many of the structures rendered evident to the eye by the microscope are, we shall scarcely wonder that many light substances are liable to come in contact with the specimen which is under examination. The cotton or flax fibres from the cloth, starch globules which adhere to the thin glass (for the small pieces are often kept in starch), portions of feathers, various kinds of hair and oil-globules are among the substances which are most frequently met with in examining different structures, and I need hardly say that their presence is purely accidental. That I am not giving needless caution upon this head, is shown by the fact that in a well known and otherwise highly valuable publication, a drawing of what is evidently a portion of feather is described as a representation of *lymphatic vessels*,—vegetable hairs are described as *nerve fibres*, and several other errors equally unpardonable occur. Now, such mistakes could only arise from utter ignorance of the characters of some of the commonest objects with which every observer ought to be very familiar. I would very strongly recommend every one to study the characters of all these substances before he attempts to make any original observations. He is sure to meet with them from time to time, and the sooner he is well acquainted with their characters the better.

The following should be very carefully examined :—

Oil globules, milk.

Potato, wheat, and rice, starch, and bread crumbs.

Portions of feathers ; worsted.

Fibres of flax ; cotton ; and silk of different colors.

Human hair, cat's hair, hair from blankets.

Fibres of wood swept from the floor, fragments of tea-leaves, hairs from plants, vegetable cellular tissue, and spiral vessels.

Particles of sand.

Many of these extraneous substances are figured in Plate LVI.

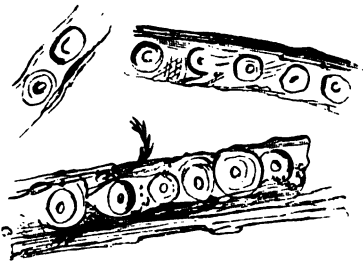
In the examination of deposits from fluid we must bear in mind the possibility of the introduction of a small quantity of one deposit into another by the pipette used for examination, and in this simple manner much difficulty and confusion may be caused to the microscopist. The pipette should be well washed immediately after it has been used, and the water which is used should be very frequently changed. In taking fluids from different bottles and other vessels the possibility of introducing various substances must be borne in mind.

**319. Of Recording Microscopical Observations.**—Taking notes of microscopical observations is a subject of great importance. The observer must endeavour to acquire the habit of describing in words the appearance of objects under the microscope. This is probably not so easy as would at first be supposed, although undoubtedly many persons are able to describe what they see much more correctly, and with greater facility, than others. Accuracy in describing microscopical specimens can only be acquired by practice, and I think it a most excellent rule to take notes of the appearances of every object submitted to examination. The time is well spent, and much of what is so described is retained in the memory. The notes should be short, and should consist of a simple statement of points which have been observed. *Inferences* should be carefully avoided, and nothing should be stated without the observer being thoroughly satisfied of its accuracy. If he is not quite certain of any observation, he should express his doubts, or place a note of interrogation after the statement. The use of indefinite terms should be avoided as much as possible, and whenever any particular word is used, a definite meaning should be attached to it. Much confusion has arisen from the use of terms which have not been well defined. Thus, the word "*granule*," by many authors, is applied to a minute particle which appears as a small speck even when examined by the highest powers, as well as to a small body with a perfectly clear centre, and with a well defined sharp outline, which would be more correctly termed a small "*globule*." So, again, the term "*molecule*" has been employed in some cases synonymously with "*granule*," but it would obviously be wrong to speak of a small globule as a molecule. It seems to me very desirable to restrict the terms "*granule*" and "*molecule*" to minute particles of matter which exhibit no *distinct structure* when examined by the highest powers at our disposal, and the term "*globule*" to circular or oval bodies of all



PLATE XVI

Fig. 256.



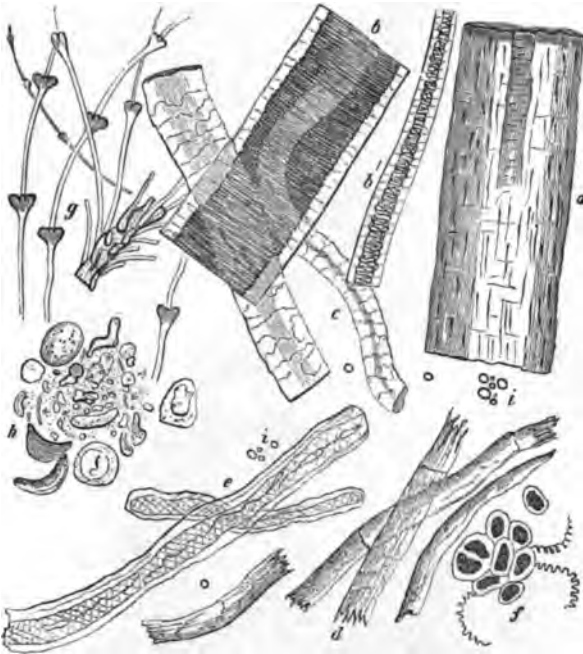
§ 318.

Fig. 257.



§ 318.

Fig. 258.



§ 318.

SUBSTANCES WHICH ARE OFTEN ACCIDENTALLY PRESENT IN MICROSCOPICAL PREPARATIONS.

Fig. 256. Fibres of deal swept from the floor.

Fig. 257. Globules of potato starch.

Fig. 258. *a.* Fragments of human hair. *b.* Cat's hair. *c.* Hair from blanket. *d.* Fibres of flax. *e.* Fibres of cotton. *f.* Fragments of tea-leaves, showing cells and spiral vessels. *g.* Portions of feather. *h.* Bread crumbs, showing wheat starch partly altered by baking and maceration. *i.* Free oil globules.

[To face page 246.]



1

sizes which have a *clear centre*, with a *well-defined dark outline*. Other examples of the use of insufficiently-defined terms might be pointed out. If an observer makes use of a term which is generally employed without any definite meaning being attached to it, he should describe at length the meaning which he assigns to it, and should, of course, use it only in this one sense.

320. **Exactness of Description** should always be aimed at, and we must remember that with a little trouble this exactness may be obtained with the use of a small number of words. That appearance of precision which is often attempted by employing long useless descriptions cannot be too much condemned. So, also, the practice of some, of describing every object in the field of the microscope without the smallest knowledge of any one of them, has been the cause of much ridicule, and has brought microscopic observation into great disrepute. Some have thought to gain the credit of being accurate observers by carefully measuring every object they see in every diameter, and putting down in numbers the results of this useless ceremony.

Such reports show that the author is thinking more of himself than his subject. He attempts to acquire a character of extreme minuteness of observation, instead of striving to advance the real interests of the science which he professes to serve—and instead of endeavouring to excite in the mind of the reader a desire for more extended knowledge and a wish to take part in a similar investigation, he perpetually gives undue prominence to himself. He who feels a real love for his subject, will try all he can to enlist others in the same cause; he will try to remove all difficulties of investigation, and endeavour to express what he has learned himself, in language which shall be intelligible to all. A certain mysterious air pervading the description of an observation,—an evident desire to coin new words,—and exaggerated statements of the importance of the facts observed, are quite misplaced where all should be clear, simple, and intelligible to every one,—and too often show indifference to the subject on the part of the author, and a want of consideration towards unlearned readers. Nothing, I believe, has been productive of so much pain and sorrow to earnest men who have devoted long lives to the prosecution of different branches of natural science, or retarded the real progress of scientific inquiry, more than that affectation of precision, and minute verbose and pompous style of description,

which has been fashionable among some microscopists, and which pervades the writings of several authorities in this imperfectly developed branch of investigation in the present day. All this is mere pretence, and not real, earnest, useful work,—distasteful to every scientific man and discouraging to every student. An extreme minuteness in description is by no means a proof of accuracy of observation. In this manner science becomes encumbered with unnecessary words, and earnest students are often intimidated when they commence investigations for themselves.

321. **Of the Importance of Making Sketches.**—Of the great importance of drawing I have already spoken. Even mere sketches in outline are of great value if the size has been correctly observed. Such sketches may be made upon ordinary smooth writing paper, cardboard, or, if preferred, on the thin tracing paper.



## TABLES FOR PRACTISING THE USE OF THE MICROSCOPE AND MICROSCOPICAL MANIPULATION.

- \*.\* All who desire to become practically familiar with the use of the microscope, and to learn how to observe, are strongly recommended to submit to the routine which a conscientious practice of the experiments given in the following Tables necessarily involves. The author is fully persuaded that the patient prosecution of the course recommended, for two or three hours, on eight different occasions, will enable the student to obtain a practical acquaintance with the elements of microscopical inquiry, which it is not possible for him to acquire by reading, or indeed, by any other plan.

TABLE I.

### ARRANGEMENT OF THE INSTRUMENT FOR OBSERVATION.—DRAWING AND MEASURING OBJECTS.

1. Arrange the microscope for examining objects by transmitted light.—§ 30, Pl. 15, Fig. 48.
2. Examine the objects upon the slide<sup>1</sup> with the inch, and afterwards with the quarter of an inch object-glasses, using first the shallow, and afterwards the deep eye-piece.—§§ 5, 6, Pl. 1, Figs. 4, 5.
3. Arrange the mirror in such a manner that the rays of light may pass through the object in a direct course or obliquely.—§ 9, Pl. 2, Fig. 10.
4. Examine the same object under the quarter of an inch object-glass with the achromatic condenser, and afterwards without the use of this instrument.—§ 32, Pl. 17, Fig. 57.
5. Draw upon paper some of the objects<sup>2</sup> on the slide.—§ 41.
  - a. Judging of the size by the eye alone.
  - b. By placing the paper on a level with the stage.
  - c. With the aid of the neutral tint glass reflector.—§ 44, Pl. 16, Fig. 51.
6. Ascertain the diameter of the objects upon the slide,<sup>3</sup> using the inch object glass and stage micrometer divided to 100ths of an inch, with the aid of the neutral tint glass reflector.—§§ 44, 62, Pl. 16, Fig. 51. Pl. 17, Fig. 59.
7. What is the magnifying power of the two French and English object-glasses on the table.<sup>4</sup>—§ 63.
  - a. With the shallow eye-piece.
  - b. With the deep eye-piece.
8. Measure the angles of the crystals<sup>5</sup> upon the slide.—§ 68, Pl. 20.

<sup>1</sup> Scales from the wing of a butterfly. <sup>2</sup> Tracheæ from a caterpillar.

<sup>3</sup> Fragments of human hair. <sup>4</sup> French quarter and one inch.—English quarter and one inch. <sup>5</sup> Crystals of cholesterine.



## TABLE IV.

ON MAKING MINUTE DISSECTIONS.—CUTTING THIN SECTIONS OF  
TISSUES FOR MICROSCOPICAL EXAMINATION.

22. Trace the nerves in the portion of tissue on the table. Pin it out on a loaded cork, and dissect it beneath the surface of water with the aid of a strong light condensed upon it by the large bull's-eye condenser, in the manner directed in § 141, Pl. 29, Fig. 121.
23. Cut some very thin sections of the different soft tissues upon the table.—§ 144.
  - a. Using the scissors.—Pl. 22, Figs. 78, 79, 80.
  - b. Using the double-edged knife.—Pl. 22, Fig. 81.
  - c. Using Valentin's knife.—Pl. 22, Fig. 82.

All these instruments must be well wetted before the section is removed.—§ 144.
24. Place some small pieces of tissue in the compressorium and dissect them under the microscope in the manner described in § 153, Pl. 30, Figs. 126, 127.
25. Make some thin sections of wood with the aid of the section cutter alluded to in § 152, Pl. 30, Fig. 125.
26. Place some of the sections of pith or bone in thin cells, cover them with thin glass, and let them be preserved as dry objects.—§ 157.
27. Ascertain the effect of the different preservative solutions upon the appearance of the sections in the microscope.—§§ 99, 100, 102, 106, 107.
28. Place some of the sections which have been allowed to soak for half an hour in the fluid in which they are to be preserved in thin glass cells, and apply the thin glass cover, observing the precautions detailed in page 93. Remove the fluid outside, and anoint the edge with Brunswick black, which must be applied with a small brush.
29. Make a thin section of the injected tissue on the table and preserve it in gelatine and glycerine.—§§ 100, 105, 106.
30. Dry another section and mount it in Canada balsam.—§165.

TABLE V.

KIDNEY.—MUSCULAR FIBRE.—PIG'S-SKIN.—PITH.—WOOD.—  
SPIRAL VESSELS.—VALLISNERIA.

31. Make thin sections of the sheep's kidney upon the table, and after washing them, subject them to examination with the inch, and afterwards with the quarter. Some may be examined in water and others in glycerine. One section should be mounted in the mixture of gelatine and glycerine.—§ 106. Observe the different characters of the tubes in the central and in the cortical portions of the organ, and endeavour to make out the following structures:—*Epithelium, basement membrane of the tubes, Malpighian bodies and capillary vessels lying between the tubes.* The arrangement of the vessels may be satisfactorily demonstrated in an injected specimen.—Table VII.
32. Take a very small fragment of the muscular fibre of the skate or eel, and after tearing it up with needles, moisten it with water, and cover it with thin glass. Endeavour to find elementary fibres in which the tube of *sarcolemma* remains entire while the *sarcous* tissue within is ruptured.—§ 136.
33. The portion of pig's-skin on the table has been allowed to dry by exposure to the air. Thin transverse sections are to be removed with a sharp knife, and subsequently moistened with water. In this manner a very thin section may be obtained, which soon regains its normal appearance. It may be mounted in any of the preservative fluids before alluded to.—§ 145.
34. Cut thin sections of the cornea and sclerotic of the eye which have been allowed to dry after having been pinned out on a board; soak them in a drop of water for twenty minutes or more, and examine them first with an inch object-glass and afterwards with a quarter.
35. Cut a thin section of the pith of the rush and examine it as a dry object; afterwards place it in fluid. Observe the air within many of the cells.
36. Demonstrate the circulation in the cells of *vallisneria spiralis*.—§ 163.
37. Wash some pieces of the sea-weed in plain water, and preserve some of them in glycerine, and others in solution of chloride of calcium.—§ 163.

## TABLE VI.

MAKING THIN SECTIONS OF BONE AND HAIR, AND MOUNTING THEM IN CANADA BALSAM.—MOUNTING DIFFERENT PARTS OF INSECTS.—SEPARATION OF DEPOSITS FROM FLUIDS.

38. Cut some thin sections of bone with the saw and grind them to the required degree of tenuity between the hones.—§ 149.
39. Upon microscopical examination they will be found covered with numerous scratches which must be removed by rubbing the sections upon a dry hone, and afterwards upon a piece of plate-glass.—§ 149.
40. When the sections of bone are sufficiently smooth, mount one of them at once in balsam, and treat another section with turpentine before immersing it in the balsam. Compare the different microscopical characters of these two specimens.—§ 166.
41. Cut some thin transverse and longitudinal sections of hair, and examine them under the quarter of an inch object-glass. These may be washed in water and mounted in Canada balsam.—§ 148.
42. After drying several portions of the insects in a capsule over the water-bath (claws, antennæ, wings, eyes, spiracles), moisten them with turpentine and mount them in Canada balsam.—§ 165.
43. After the deposit suspended in the fluid in the conical glass has subsided,<sup>1</sup> a portion is to be removed with the pipette and placed in a cell, or in the animalcule cage, for examination.—§ 173, Pl. 35, Fig. 157, Pl. 11, Fig. 32.
44. The fluid may then be allowed to evaporate spontaneously or by placing the slide under a bell-jar over sulphuric acid, and the residue mounted in Canada balsam.
45. Subject some of the infusoria in the specimen of water on the table to examination with a quarter of an inch object-glass.<sup>2</sup>—§ 175.

<sup>1</sup> Small marine shells, sand, &c.

<sup>2</sup> Water containing portions of vegetables which had been kept for several days.



## TABLE VII.

ON INJECTING WITH OPAQUE AND TRANSPARENT MATERIALS.—  
PRUSSIAN BLUE FLUID FOR INJECTION.

46. Arrange the injecting apparatus conveniently (§ 204) and proceed to inject the artery supplying the eye-ball of the ox's eye on the table, with size and chromate of lead.—§§ 185, 188.
47. *Eye*.—Introduce the pipe into the vessel running close to the large optic nerve, and tie it carefully, observing the precautions detailed in § 204. The eye must be allowed to remain in warm water until warm through, and the injecting material prepared in the manner described; it is to be mixed with melted size and strained immediately before use. When the injection is complete the eye is to be placed in cold water. Should it become very much distended by the accumulation of the injection within it, a puncture may be made in the cornea, which will permit the escape of the aqueous humour, and then the vessels may be more completely injected.—§ 204.
48. Prepare some Prussian blue injecting fluid.—§ 196.
49. *Frog*.—Insert an injecting pipe into the aorta of the frog in the manner described in § 204, and slowly inject the fluid.
50. The specimens having been completely injected portions may be submitted to microscopical examination.—§ 205.
51. The globe of the eye may be opened and portions of the following tissues removed with scissors, *ciliary processes* situated behind the iris, the *retina* (the most internal of the membranes within the globe), the *choroid* (external to the delicate retina). These, after having been carefully washed in water, may be submitted to examination in fluid with the inch object-glass.  
The ciliary processes and the choroid require to be well washed in order to remove the black pigment with which they are covered.
52. Portions of the lung and intestines of the frog may be removed, and after being well washed, may be submitted to examination. These are to be examined by transmitted light, and may be placed in glycerine. The inch object-glass should be employed in the first instance, and afterwards the quarter.



## TABLE VIII.

## OF THE USE OF CHEMICAL REAGENTS IN MICROSCOPICAL INVESTIGATION.

53. Test the powder on the glass slide for the presence of carbonate,<sup>1</sup> using the precautions detailed in § 239.
54. Each of the solutions<sup>2</sup> is to be diluted and separately tested for sulphates, phosphates, and chlorides.—§ 239.
55. Make some crystals of common salt.
  - a.* By evaporating a solution rapidly to dryness on a glass slide.
  - b.* By allowing the solution to evaporate slowly until crystals form, when a thin glass cover may be applied and the crystals subjected to microscopical examination.—§ 244, Pl. 43, Fig. 200.
56. Fill one of the little bottles with capillary orifices with acetic acid.—§ 236.
57. Examine some of the white fibrous tissue<sup>3</sup> under a quarter, before and after the addition of a drop of acetic acid.—§ 227.
58. Ascertain the effect of a solution of caustic soda upon the cells on the slide.<sup>4</sup>—§ 230.
59. Describe the microscopical characters of the structures upon the glass slide,<sup>5</sup> and sketch roughly their most important characters.—§§ 57, 320.
60. What is the nature of the substances forming the deposit in the glass.<sup>6</sup>—§ 318.

<sup>1</sup> Chalk.<sup>2</sup> Sulphate of soda, phosphates of lime, and ammonia and magnesia, and common salt dissolved in water to which a few drops of nitric acid have been added.<sup>3</sup> The white tendon of a muscle of any small animal, as a mouse, &c.<sup>4</sup> Cuticle.<sup>5</sup> Eye and proboscis of a common fly.<sup>6</sup> Potato-starch, blanket-hair, portions of feathers.

## APPARATUS REQUIRED IN MICROSCOPICAL INVESTIGATION.

### I.—The Microscope.

#### NECESSARY.

1. *Microscope* with large stage, firm tripod stand, coarse and fine adjustments, double mirror, and arrangement for inclining body; generally termed the *Student's Microscope*.—§ 15, Pl. 3, 4, 5.

The student's microscope with two powers and bull's-eye condenser costs from five to ten guineas.

2. Pocket Clinical or Field Microscope, in case, with pipettes, test tubes, &c. § 21.
3. *Object-glasses*.—1. *The inch* magnifying from 30 to 40 diameters, the glasses of which can be removed one by one, so that lower powers can be obtained.
2. *The quarter of an inch* magnifying about 200 diameters. These glasses should *define well*, the field should be *perfectly flat* and free from *coloured fringes*, and they should admit a sufficient amount of light.—§ 6, Pl. 1, Fig. 5.

#### ADVANTAGEOUS.

*Large Microscope* provided with moveable stage and all the modern improvements.—§ 17.

With two powers, this instrument costs from 20 to 30 guineas.

*Microscope for Traveling*.—§ 19.

*Binocular Microscope*. § 18.

*Two-inch object-glass*. § 6.

*Eighth of an inch*.—§ 6.

*Twelfth of an inch*.

*Twenty fifth*.—§ 305.

### II.—Accessory Apparatus.

4. *Diaphragm plate*.—§ 13, 31, Pl. 2, Fig. 9.
5. *Bull's-eye condenser*.—§ 25, Pl. 16, Figs. 49, 50.
- Gillet's achromatic condenser*.—§ 33.
- Polariscope*.—§ 36, Pl. 20, Fig. 69.
- Spot glass*.—§ 29, Pl. 17, Fig. 58.

*For Artificial Illumination.*

## NECESSARY.

6. Small French moderator lamp.—§ 39.

## ADVANTAGEOUS.

Smith & Beck's camphine lamp, or Mr. Highley's gas lamp.  
§ 39, Pl. 16, Fig. 55.

## III.—Apparatus for Drawing Objects.

7. *Neutral tint glass reflector*.—§ 44, Pl. 16, Fig. 51.  
8. Common hard pencils, steel pens, Indian ink, fine Bristol board, smooth white paper.

## IV.—Apparatus for Measuring Objects and for Ascertaining the Magnifying Power of the Object Glasses.—§§ 58 to 66.

9. *Stage micrometers* divided into 100ths and 1000ths of an English inch.—§ 60. Nobert's lines, which may be used also as *test objects*.—§ 61.  
10. Maltwood's finder.—§ 67.

## V.—Instruments and Apparatus for General Purposes.

11. *Wire retort stand*.—§ 70, Pl. 21, Fig. 72. *Water bath*.—§ 73, Fig. 76.  
12. *Tripod wire stands*.—§ 71, Pl. 21, Figs. 75, 77.  
13. *Spirit lamp*.—§ 69, Pl. 21, Fig. 73.  
14. *Evaporating basins*.  
15. *Watch glasses*.—§ 85.  
16. *Thin glass*.—§ 84.  
17. *Plate-glass slides*.—§ 83.

## VI.—Instruments for Making Dissections and for Cutting Thin Sections of Soft Tissues.

18. *Common scalpels*.—§ 74. *Valentin's knife*.—§ 77,  
18a. *Double-edged scalpel*.—§ 75, Pl. 22, Pl. 22, Figs. 82, 83.  
Fig. 81.  
19. *Scissors*.—Ordinary form and two small pair, one with curved blades.—§ 79, *Spring scissors*.—§ 79, Pl. 22, Figs. 78, 79, 80. Pl. 22, Fig. 80.  
20. *Needles mounted in handles*.—§ 80, *Compressorium*.—Fig. 84. § 153, Pl. 30, Fig. 126, 127.  
20a. *Needles flattened near the points*.—§ 80.

## NECESSARY.

## ADVANTAGEOUS.

21. *Forceps*.—One pair of ordinary dissecting forceps, and one pair with curved blades.—§ 81, Pl. 23, Figs. 85, 86.

*For Dissecting under Water.*

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|--|---|
| 22. <i>Glass dishes</i> of various sizes from an inch to two inches in depth.—§ 141. | <i>Large bull's-eye condenser</i> , for condensing a strong light upon the object.—§ 142, Pl. 29, Fig. 121. |
| 23. <i>Loaded corks</i> .—§ 142, Pl. 30, Fig. 124.                                   |   |
| 24. <i>Fine pins</i> and <i>thin silver wire</i> .                                   |   |
| 25. <i>Tablets of wax</i> and <i>gutta percha</i> .—§ 143.                           |   |

*For Cutting Thin Sections of Hard Tissues.*

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|--|---|
| 26. <i>Saw</i> with fine teeth, for cutting thin sections of bone.—§ 149, Pl. 23, Fig. 90. | <i>Section cutter</i> for cutting thin sections of wood.—§ 152, Pl. 30, Fig. 125. |
| 27. <i>Hones</i> for grinding the sections thinner and polishing them.—§ 149.              |   |
| 28. <i>Strong knife</i> for cutting thin sections of horn, &c.—§ 147, Pl. 23, Fig. 89.     |   |

## VII.—Cements.

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|--|--------------------------------------|
| 29. <i>Brunswick black</i> , containing a few drops of a solution of India rubber in coal naphtha.—§ 91. | <i>Gold Size</i> .—§ 87.             |
| 29a. <i>Bell's cement</i> .—§ 90.  | <i>Solution of shell-lac</i> .—§ 59. |
| 30. <i>Marine glue</i> .—§ 92.   |                                      |
| 31. <i>Gum water</i> .—§ 97.   |                                      |
| 32. <i>Gum</i> thickened with <i>starch</i> or <i>whiting</i> .—§ 97.                                    |                                      |
| 33. <i>French cement</i> , composed of lime and India rubber.—§ 98.                                      |                                      |

## VIII.—Preservative Fluids.

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|--|--|
| 34. <i>Spirit and water</i> .—§ 99.                  | <i>Gelatine and glycerine</i> .—§ 106. |
| 35. <i>Glycerine</i> .—§ 100.                        |  |
| 36. <i>Solution of naphtha and creosote</i> .—§ 102. | <i>Gum and glycerine</i> .—§ 107.      |
| 37. <i>Chromic acid</i> .—§ 104.                     |  |
| 38. <i>Turpentine</i> .                              |  |
| 39. <i>Canada balsam</i> .—§ 94.                     |  |



## NECESSARY.

## ADVANTAGEOUS.

**IX.—Apparatus Required for Making Cells and for Cutting and Grinding Glass.**

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|--|--|
| <p>40. <i>Brass plate</i> for heating slides to which marine glue is to be applied.—§ 72, Pl. 21, Fig. 74.<br/> <i>Cements</i> before enumerated.—§§ 87 to 98.</p> <p>41. <i>Small brush</i> made of bristles.</p> <p>42. <i>Tin foil</i> of different degrees of thickness.—§ 118, Pl. 24, Fig. 96.</p> <p>43. <i>Writing diamond</i>.—§ 120, Pl. 24, Fig. 99.</p> <p>44. <i>Glazier's diamond</i>.—§ 119, Pl. 24, Fig. 98.</p> <p>45. <i>Flat stone or pector plate</i> for grinding glass.—§ 121.</p> <p>46. <i>Emery powder</i>.</p> <p>47. <i>Old knife</i> and small chisel for cleaning off superfluous glue.—§ 123, Pl. 24, Fig. 94.</p> <p>48. <i>Solution of potash</i> (liquor potassæ).</p> <p>49. <i>Sections of glass tubes</i> and of thick square vessels, of various sizes, for making cells for the preservation of injections.—§ 127, Pl. 25, Figs. 100, 104.</p> | <p><i>Shadbol's apparatus</i>.—§ 116, Pl. 24, Fig. 93.</p> <p><i>Brass rings</i> for mounting circles of thin glass.—§ 120, Pl. 24, Fig. 94.</p> <p><i>Wooden forceps</i> for holding glass slides.—§ 82.</p> <p><i>Shallow concave glass cells</i>.</p> <p><i>Moulded glass cells</i>.—§ 130.</p> |
|--|--|

**X.—Apparatus for Preserving Objects in Air, Fluid, and Canada Balsam.**

- |  |   |
|--|---|
| <p>50. <i>Cells</i> of various sizes, before enumerated.—§ 126, Pl. 25, Fig. 105.<br/> <i>Brunswick Black</i>.<br/> <i>Gum</i> thickened with whiting.—§ 97.</p> <p>51. <i>Thin glass</i> cut of the requisite size.<br/> <i>Preservative solutions</i>.—§§ 99 to 113.</p> <p>52. <i>Watch glasses</i> to soak sections in the preservative fluids.—§ 85.</p> <p>53. <i>Glass shades</i> to protect recently mounted preparations from dust.—Note, § 159, Pl. 27, Fig. 92.</p> <p>54. <i>Brass plate</i>.—§ 72, Pl. 21, Fig. 74.<br/> <i>Canada balsam</i>.—§ 94.<br/> <i>Needles</i> to remove air bubbles.</p> | <p><i>Apparatus</i> for pressing down the thin glass cover while the cement is drying.—§ 161, Pl. 35, Fig. 159.</p> <p><i>Bell jar</i> with vessel for sulphuric acid, covered with wire gauze.</p> <p><i>Air-pump</i> to remove air bubbles from the interstices of a tissue.—§ 165, Pl. 35, Fig. 160.</p> |
|--|---|

## NECESSARY.

## ADVANTAGEOUS.

**XI.—Apparatus Required for the Separation of Deposits from Fluids and for their Preservation.**

55. *Conical Glasses*.—§ 171, Pl. 37, Fig. 171. *Glass troughs for Zoophytes*.—p. 46.  
 56. *Pipettes*.—§ 172, Pl. 37, Fig. 172.  
 57. *Wash-bottle*.—§ 180, Pl. 37, Fig. 173.  
 58. *Cells for the examination of infusoria*.—§ 175, Pl. 35, Fig. 157, Pl. 11, Fig. 32.  
 59. *Animalcule cage*.—§ 133, Pl. 35, Fig. 157, Pl. 40, Fig. 32.

**XII.—Instruments and Apparatus Required for Making Injections.**

60. *Injecting syringe*, holding from half an ounce to an ounce.—§ 183, Pl. 38, Fig. 184, and Fig. 179.  
 61. *Pipes* of various sizes.—§ 183, Pl. 38, Fig. 182. *Stop-cocks*.—§ 183, Pl. 38, Fig. 182.  
 62. *Corks* for stopping the pipes.—§ 183, Pl. 38, Fig. 177.  
 63. *Needle* for passing the thread round the vessel.—§ 183, Pl. 38, Fig. 183.  
 64. Thread of different degrees of thickness.  
 64. *Bull's-nose forceps* for stopping vessels.—§ 183, Pl. 38, Fig. 178.

*For Making Opaque Injections.*

65. *Size* or *gelatine*.—§ 185. *Injecting can*, made of copper.—§ 184, Pl. 38, Fig. 180.  
 66. *Vermilion*.—§ 187.  
 67. *Bichromate of potash* and *acetate of lead* for making solutions for precipitating *yellow chromate of lead*.—§ 188.  
 68. *Carbonate of soda* and *acetate of lead* for making solutions for precipitating *carbonate of lead*.—§ 189.

*For Making Transparent Injections.*

69. *Ferrocyanide of potassium*. "*Muriated Carmine*.—§ 195. *tincture of iron*." *Glycerine* and *Spirits of wine* for preparing the *Prussian blue injecting fluid*.—§ 196.

## NECESSARY.

## ADVANTAGEOUS.

**XIII.—Chemical Analysis in Microscopical Investigation.**

- |   |                                |
|---|--------------------------------|
| 70. <i>Platinum foil.</i>   | <i>Small platinum capsule.</i> |
| 71. <i>Test tubes and rack.</i> —Pl. 41, Fig. 192.                    | <i>Small flasks.</i>           |
| 72. <i>Small tubes</i> about an inch or an inch and a half in length. | <i>Platinum wire.</i>          |
| 73. <i>Stirring rods.</i>   |                                |
| 74. <i>Evaporating basins.</i> —Pl. 21, Fig. 76.                      |                                |
| 75. <i>Watch glasses.</i> —§ 85.                                      |                                |
| 76. <i>Small glass bottles with capillary orifices.</i>               |                                |
| —§ 236.   |                                |
| 77. <i>Wire triangles, tripods.</i> —§ 71, Pl. 21.                    |                                |
| 78. <i>Small retort stand.</i> —§ 70, Pl. 21.                         |                                |

**Reagents:—**

79. *Alcohol.*—§ 219.  
 80. *Ether. Chloroform.*—§ 220.  
 81. *Nitric acid.*—§ 223.  
 82. *Sulphuric acid.*—§ 223.  
 83. *Acetic acid.*—§ 225.  
 84. *Hydrochloric acid.*—§ 224.  
 85. *Ammonia.*—§ 231.  
 86. *Solution of potash.*—§ 228.  
 87. *Solution of soda.*—§ 229.  
 88. *Nitrate of silver.*—§ 233.  
 89. *Nitrate of Barytes.*—§ 232.  
 90. *Oxalate of ammonia.*—§ 234.  
 91. *Iodine solution.*—§ 235.  
 92. *Test Papers.*

**XIV.—Cabinet for Preserving Microscopical Specimens.**

93. *Drawers* arranged so that the specimens may lie perfectly flat.—§ 181.

## BRITISH MICROSCOPE MAKERS.

Baker, 44, High Holborn, London.  
 Bryson, Princes-street, Edinburgh.  
 Collins, Charles, 77, Great Titchfield-street,  
 Oxford-street.  
 Crouch, H. and W., Regent's Canal, Com-  
 mercial-road, London.  
 Dancer, 43, Cross-street, Manchester.  
 Field, Birmingham.  
 Highley, S., Green-street, Leicester-  
 square, London.  
 King, Bristol

Ladd, W., 12, Beak-st., Regent-st., London.  
 Murray and Heath, 43, Piccadilly, London.  
 Parkes and Son, St. Mary's-row, Bir-  
 mingham.  
 Pillscher, 88, New Bond-street, London.  
 Powell and Lealand, 170, Euston-road,  
 London.  
 Ross, 2, Featherstone-buildings, London.  
 Salmon, 100, Fenchurch-street, London.  
 Smith, Beck, and Beck, 6, Coleman-street,  
 London.

## FOREIGN MICROSCOPE MAKERS.

Amici, Modena.  
 Bénéche, Berlin, Tempelhofer Strasse 7.  
 Brunner, Paris.  
 Chevallier, Paris.  
 Hartnack and Oberhäuser, Place  
 Dauphine 21, Paris.  
 Hasert, E., Eisenach.  
 Kellner, Wetzlar.

Merz, G. and S., Munich.  
 Mirand, A., senr., Paris.  
 Nachet, Rue St. Severin 17, Paris.  
 Ploesl, S., Vienna.  
 Schröder, Hamburg, Holländischer  
 Brook 31.  
 Schiek, F. W., Berlin, Halle'sche Str. 15.  
 Zeis, C., Jena.

Most of the microscope makers furnish cabinets and boxes for objects, apparatus and instruments required by the microscopist.

## PREPARERS OF MICROSCOPIC OBJECTS.

Barnett, J. E., Whitehall-st., Tottenham.  
 Hett, A., 4, Albion-grove, Islington.  
 Hudson and Sons, Greenwich.  
 Norman, J., 178, City-road, London, E.C.

Topping, C. M., 7, Haverstock-street,  
 City-road.  
 Wehh, H., George-street, Balsall-keath,  
 Birmingham.

Collections of objects of various kinds may also be obtained of almost all the microscope makers.

## MATERIALS AND APPARATUS FOR MOUNTING OBJECTS.

Crouch, Regent's Canal, Commercial-  
 road, London.  
 Griffin, 119, Bunhill-row, London.  
 Highley, Green-st., Leicester-sq., London.

Matthews, Portugal-street, Lincoln's-inn,  
 London.  
 Smith, Beck, and Beck, Cornhill, London.  
 Norman, 178, City-road, London, E.C.

## ARTISTS. DRAUGHTSMEN.

Dr. Westmacott, King's College, London.  
 Aldous, W. Lens, 47, Liverpool-street,  
 King's Cross.

West, Tuffen, W. West, Hatton-garden.  
 Searson, J., Royal College of Surgeons.

## WOOD ENGRAVERS.

Hart, Mr., 33A, Red Lion-square, E.C.  
 Powell, Miss, 170, Euston-road, N.W.

Ruffe, Mr., 17, Princes-road, Kennington.  
 Stevens, Mr., 48, Essex-st., Strand, W.C.

## PRINTERS. LITHOGRAPHIC AND PHOTOGRAPHIC PRINTERS.

Aldard, 22½, Bartholomew-close.  
 Blanchard and Sons, Millbank-street.  
 Harrison and Sons, St. Martin's-lane.  
 West, W., Hatton-garden, E.C.

Brooks, V., Lithographic Printer, Photo-  
 graphic Lithography, 1, Chandos-street,  
 Covent-garden.  
 Toovey, Photographic Lithographer.

## LITHOGRAPHIC STONES—DIAMONDS FOR ENGRAVING, AND OTHER APPARATUS USED IN LITHOGRAPHY.

Hughes and Kimber, West Harding-street,  
 E.C.

Stoer Brothers, Vulcan Wharf, 16, Earl-  
 street, Blackfriars, London.

## APPARATUS FOR DRAWING, ENGRAVING, &amp;c.

PENCILS, COLOURS, TRACING PAPERS, &c.

Brodie, Long-acre, and other Artists' Colourmen.



# WORKS ON THE MICROSCOPE, &c., USEFUL TO THE STUDENT.

The Microscope and its Revelations. Dr. W. B. Carpenter, F.R.S. John Churchill and Sons. 1862.

The Microscope. Prof. Quekett. Baillière.

The Microscope; its History, Construction, and Teachings. Jabez Hogg.

The Microscope in Vegetable Physiology. Schacht.

The Microscope. Hannover, translated.

The Microscope. Dr. Lardner.

The Microscope. Dr. Wythes.

Manual of Human Microscopic Anatomy. Prof. Kölliker. Translation by Dr. Chance.

The Microscope in its Application to Clinical Medicine. Dr. Lionel Beale, F.R.S. 1858. Churchill and Sons.

The Microscopic Anatomy of the Human Body in Health and Disease. A. H. Hassall, M.D.

On the Structure and growth of Tissues. Dr. Lionel Beale, F.R.S. 1861. Churchill and Sons.

Text Book of the Microscope. Dr. Griffith, F.L.S. 1864. John Van Voorst.

Text Book of Objects for the Microscope. J. Lane Clarke.

The Preparation and Mounting of Microscopic Objects. Thomas Davies. Robert Hardwicke.

Micrographic Dictionary Griffith and Henfrey.

Microscopic Teachings. The Hon. Mrs. Ward. Groombridge and Sons.

Half-hours with the Microscope. Dr. Lankester, F.R.S.

Evenings at the Microscope. P. H. Gosse, F.R.S.

Sea Side Studies. G. H. Lewis.

A Manual of the Sub Kingdom Protozoa. J. R. Greene, B.A. Longmans. 1863.

A Manual of the Sub Kingdom Cœlenterata. J. R. Greene, B.A. Longmans. 1863.

A History of Infusoria, including the Desmidiæ and Diatomaceæ. Dr. Andrew Pritchard. Whitaker and Co.

British Diatomaceæ. The Rev. W. Smith.

British Freshwater Algae. A. H. Hassall.

- Marvels of Pond Life. H. J. Slack. Groombridge and Sons.  
 Butterfly Vivarium. Noel Humphreys.  
 The Common Objects of the Microscope. The Rev. J. G. Wood. Routledge and Co.  
 The Common Objects of the Country. The Rev. J. G. Wood. Routledge and Co.  
 The Common Objects of the Sea Shore. The Rev. J. G. Wood. Routledge and Co.  
 The Aquarium, of Marine and Freshwater Animals and Plants. G. B. Sowerby, F.R.S. Routledge and Co.  
 British Seaweeds. With Notices of some of the Freshwater Algae. The Rev. D. Landsborough. Routledge and Co.

## FOREIGN BOOKS.

- Das Mikroskop. P. Harting and Dr. F. W. Theile. Vieweg and Sohn. 1859.  
 Das Mikroskop und die Mikroskopische Technik. Dr. Heinrich Frey. 1863.  
 Das Mikroskop und sein Gebrauch für den Arzt. Dr. Hermann Reinhard. 1864.  
 Beiträge zur Neuern Mikroskopie. Fried Reinicke. 1862.  
 Gewebelehre. Gerlach.  
 Lehrbuch der Histologie. Leydig.  
 Du Microscope et des Injections. Robin. 1849.  
 Observateur au Microscope. Dujardin. 1842.

## JOURNALS, PERIODICALS.

- Quarterly Journal of Science. Edited by J. Samuelson and W. Crookes, F.R.S. John Churchill and Sons.  
 Quarterly Journal of Microscopical Science. Edited by Dr. Lankester, F.R.S., and George Busk, F.R.S. John Churchill and Sons.  
 The Reader. Weekly.  
 Popular Science Review. Edited by Prof. Henry Lawson, M.D. Hardwicke.  
 Archives of Medicine. Edited by Dr. Lionel Beale, F.R.S. John Churchill and Sons.  
 Intellectual Observer. Monthly. Groombridge and Sons.  
 The Electrician. Weekly. Saunders and Ottley.



# INDEX.

- ABERRATION, spherical and chromatic, 7.
- Achromatic condenser, 23.
- Acid, acetic, 130.
- syrup, 202.
- and glycerine, for washing and preserving thin sections, 202.
- action of, upon animal structures, 121.
- in examination of the spinal cord, 146.
- chromic, 61.
- nitric, 132.
- Acids, effects of, upon animal structures, 131.
- Achromatic condenser, 23.
- Action of glycerine and syrup on tissues, 198.
- Adipose tissue, 94.
- Adjustments for altering the focus, 8.
- Air-bubbles, 100.
- Air, removal of, from the interstices of a tissue, 100.
- Alcohol, 58.
- effects of, in hardening tissues, 130.
- Alkalies, effects of, upon animal structures, 133.
- Alum, solution of, 63.
- Ammonia, 134.
- Ammonia-magnesian phosphate, 139.
- Analyzer, 24.
- Anatomical peculiarities of tissues, 78.
- Angle of aperture, 7.
- Anilin colours, 192.
- Animalcule cage, 72, 86.
- Apparatus for cutting thin sections of wood, 85.
- necessary for microscopical research, 12, 19, 241.
- Arranging light, 27.
- Arsenious acid, solution of, 63.
- Arsenuretted hydrogen, 63.
- Artificial illumination, 25.
- Artificial injections, 55.
- Artists, engravers, &c., 263.
- BALSAM, Canada, 55.
- solutions of, 56.
- Barytes, nitrate, 135.
- Bell's cement, 53.
- Binoocular microscope, 13.
- Blanket hair, 245.
- Blood corpuscles, 101.
- Blue colours for staining, 192.
- injections, Prussian blue, 114.
- Boiling tissues, 145.
- Bone, examination of, 84.
- Books on the Microscope, British and foreign, 264.
- Brass plate, 48.
- Bread crumbs, 74.
- Brunswick black, 54.
- BYRON, microscope maker, 263.
- Built glass cells, 70.
- Bull's-eye condenser, 21.
- BURNETT'S solution, 63.
- CABINETS for microscopic preparations, 105.
- Camera lucida, 27.
- photographic, 163.
- Canada balsam, 55.
- solutions of, 56.
- mounting preparations in, 98.
- examination of objects in, 98.
- of hard tissues in, 99.
- Canaliculi of bone, 99.
- Cans for injecting, 109.
- Capillary vessels, 107.
- Carbolic acid, solution of, 61.
- Carbonate, testing for, 139.
- of lead, 110.
- of lime by reflected light, 87.
- in different media, 87.
- Carmine injecting fluid, 112.
- fluid adapted for researches with the aid of the highest powers, 201.
- Caution necessary in drawing inferences from microscopical examination, 241.
- Cells, 223.
- for preserving preparations, 65.
- for examination of deposits, 72.
- Brunswick black, 66.
- gutta percha, 71.
- marine glue, 66.
- paper, 66.
- tinfoil, 66.
- very thin glass, 68.
- Cements, 53.
- French, composed of lime and India-rubber, 56.
- for attaching gutta percha to the glass slides, 54.
- for attaching cover to large glass cells, 56.
- Chemical analysis in microscopical investigation, 123.
- apparatus required for, 128.
- reagents, use of in elucidating structures, 123.



- Chemical reagents dissolved in glycerine, 202.  
 — tests, application of, to objects under the microscope, 137.  
 CHEVALIER, microscope maker, 263.  
 Chloride of calcium, solution of, 63.  
 Chromic acid, 61.  
 Chromate of lead, 110.  
 Chromatic aberration, 7.  
 Ciliary motion, 80.  
 — in the kidney of the frog and newt, 81.  
 Ciliated epithelium, 80.  
 CLARKE, Mr. J. L., method of examining spinal cord, 146.  
 Cleanliness, importance of, in microscopical investigation, 241.  
 Cobweb micrometer, 35.  
 Colouring matters used for injection, 110.  
 — solutions, various, with glycerine, 202.  
 — chromate of lead, 110.  
 — Prussian blue, 113.  
 — vermilion, 110.  
 Compressorium, 85.  
 Concave glass cells, 70.  
 Condenser, achromatic, 23.  
 — bull's eye, 21.  
 — GILLET's, 24.  
 Conical glasses, 101.  
 Corks for dissecting under water, 81.  
 — for injecting pipes, 108.  
 Cotton fibres, 245.  
 Cover, application of, to cell, 93.  
 Covering glass, very thin, adapted for very high powers, 220.  
 Creosote solution, 60.  
 Crystals, on measuring the angles of, 43.  
 — formation of, 140.  
 Cutting glass, 67.  
 — circular pieces of thin glass, 67.  
 — thin sections of soft tissues, 83.  
 — hard tissues, 84.  
 DANCER, microscope maker, 263.  
 Dark-ground illumination, 21.  
 Day light, examining microscopical objects by, 20.  
 DEANE, Mr., his preservative gelatine, 61.  
 — made with blowpipe, 71.  
 DELVER, Mr., on photographs, 150.  
 Demonstrating minute structure with the highest powers, 197.  
 DENNIS, Mr., on glass cells, 70.  
 Deposits, collecting small quantities of, 102.  
 Destroying the life of animals intended for injection, 122.  
 Diamond, cutting, 67.  
 — writing, 67.  
 Diaphragm, 10, 23.  
 Diatomaceae, siliceous skeletons of, 101.  
 Dissecting microscopes, 14, 17.  
 — tissues in viscid media for examination with high powers, 204.  
 Dissection under the surface of fluid, 81.  
 Draughtsmen, artists, and engravers, 263.  
 Drawing objects, 26.  
 — on transfer paper, 29.  
 — on stone, 30.  
 Drawings intended to be engraved, 28.  
 Drying tissues for microscopical examination, 63.  
 ENGRAVERS, 263.  
 Epithelium, ciliated, 80.  
 Ether, 129.  
 Evaporation and drying, 48.  
 Examination of deposits from fluid, 101.  
 — of objects by reflected light, 20.  
 — of objects by transmitted light, 28.  
 — of organs in lower animals, 79.  
 — of soft tissues, 93.  
 — of hard tissues, 84, 99.  
 — of vegetable tissues, 55.  
 — of circulation in the cells of valvuliferous, 96.  
 — of substances in different media, 87.  
 — of tissues, 78.  
 — of muscle, 93.  
 — of tissues by boiling previously, 145.  
 — of tissues by drying, 31.  
 — of the same object in different media, 87.  
 — of tissues, magnifying powers required in, 6.  
 — of crystals, 142.  
 Extraneous substances, 245.  
 Eye-pieces, 6.  
 Eye piece micrometer, 35.  
 FALLACIES to be guarded against, 241.  
 Fibres of deal wood, 245.  
 — and membranes produced artificially, 243.  
 Fibrille of muscle, 77.  
 Finders, 42.  
 Fish, injecting, 116.  
 Fixing cells to glass slides, 65.  
 — cover on large glass cells, 71.  
 Flax, 245.  
 Flesh, examination of, 89.  
 Fluid for injecting, preparation of, 107.  
 Focus, arrangement for altering, 8.  
 Forceps, 51.  
 — for stopping vessels in injecting, 109.  
 Formed material, 214, 223.  
 FRAUNHOFER, microscope maker, 263.  
 FREEE, Dr., his method of making thin glass cells, 68.  
 French cement composed of lime and India-rubber, 56.  
 Frog, injection of, 118.  
 Fungi, 101.  
 GAS lamp, 25.  
 Gelatine, preservative, 61.  
 — and glycerine, 62.  
 — and size for injecting, 109.  
 Generalization in microscopical inquiries, 240.  
 GERLACH, his carmine injecting fluid, 112.  
 Germinal matter, 214, 223.  
 GILLET's condenser, 24.  
 Glands, injection of, 119.  
 Glass cells, 6.  
 — cover, very thin, for the highest powers, 220.  
 — for pollishing thin sections of bone, &c., 84.

- Glass slides, 51.  
 Glasses for dissecting under water, 81.  
 Globule, 100.  
 Glycerine, 58.  
 — action of, on tissues, 198.  
 — and acetic acid for washing and preserving thin sections, 202.  
 — chemical reagents dissolved in, 202.  
 — and syrup, 200.  
 GOADBY, Dr., preservative solution, 62.  
 Gold size, 53.  
 Goniometer, 43.  
 Granule, 246.  
 Grinding glass, 67.  
 — thin sections of hard tissues, 84.  
 Gum, 56.  
 — and glycerine, 62.  
 Gutta percha cement, 54.  
 GUY, Dr., his cells, 72.
- HAIR, transverse and longitudinal sections of, 84.  
 Hard tissues, practical operation of preparing, for examination with the highest powers, 209.  
 Hardening tissues, 83.  
 — solutions for, 144.  
 Heating objects, apparatus for, 129.  
 HEREFATH, Dr., on crystals of iodo-quinine, 24.  
 High magnifying powers, use of, 215.  
 Highest magnifying powers, 218.  
 HIGLEY, microscope maker, 263.  
 Holder for pressing objects between glasses, 66.  
 Bones for grinding hard tissues, 84.  
 Horn, sections of, 83.  
 How to examine an object in the microscope, 73.  
 Huyghenian eye-piece, 6.
- ICELAND spar, 24.  
 Igniting substances to remove organic matter, 127.  
 Illumination, artificial, 25.  
 — dark ground, 21.  
 — of objects magnified by very high powers, 220.  
 — of opaque objects, 21.  
 — of transparent objects, 23.  
 Importance of observers delineating their own work, 32.  
 — of examining objects in various ways, 80.  
 Inclining microscope, arrangement for, 9.  
 Increasing the size of the image without altering the object-glass, 221.  
 Influence of media upon the microscopical appearance of substances, 80.  
 Infusoria, examination of, 103.  
 Injecting, 107.  
 — cans, 109.  
 — coloring matters employed in, 110.  
 — fishes, insects, mollusca, 117.  
 — fluids adapted for researches with the aid of the highest powers, 200.  
 — lymphatic vessels, 121.  
 — operation of, 117.  
 — pipes, 108.  
 — syringes, 108.  
 — Prussian blue fluid for, 114.
- Injections, natural and artificial, 108.  
 — mounted in Canada balsam, 122.  
 — of liver, 120.  
 Ink, lithographic, 31.  
 Instruments, 49.  
 Iodo-quinine, 24.
- JACKSON, Mr., his micrometer, 35.
- KIDNEY of newt, 79.  
 Knives, dissecting, 49.  
 — section, of new form, 49.  
 — thin-bladed, 49.  
 — Valentin's, 49.
- LACUNE of bone, 100.  
 LADD, microscope maker, 263.  
 Lamp, HIGLEY's gas microscope, 25.  
 — camphine, SMITH and BECK's, 25.  
 — spirit, 47.  
 LIEBERKUH, 21.  
 Light, polarized, 24.  
 — reflected, examination by, 20.  
 — transmitted, 23.  
 Lime, carbonate of, 87.  
 Lime, and India rubber cement, 56.  
 — phosphate of, 139.  
 Lithographers,  
 Lithographs, obtaining of mic. drawings, 29.  
 Lithographic ink, 31.  
 Loaded corks, 81.  
 Lymphatic vessels, 76.  
 — injecting of, 121.  
 — feathers mistaken for, 263.
- MAGIC LANTERN, photographs of microscopic objects for, 187.  
 Magnifying power, method of ascertaining, 38.  
 — use of very high, 215.  
 MALTWOOD, Mr., his finder, 42.  
 Marine glue, 54.  
 MATTHEWS, Mr., new form of Valentin's knife, 50.  
 Matters, extraneous, 245.  
 Measuring, method of, 37.  
 — objects with the camera, &c., 37.  
 Measurements, table for conversion of British and Foreign, 40.  
 Media in which tissues should be placed for examination, 89.  
 Mercurial injection, syringe for, 116.  
 MENZ, microscope maker, 263.  
 Metallic reflector, 21.  
 Methylated spirits of wine, 68.  
 Method of examining soft tissues, 78.  
 — of rendering tissues hard and transparent, 146.  
 — of increasing the size of the image without altering the object-glass, 221.  
 — of preparation adapted for researches with the aid of the highest powers, 193.
- Micrometer, 35.  
 — cobweb, 35.  
 — eye-piece, 35.  
 — stage, 36.
- Microscope, 4.  
 — body of, 9.  
 — diaphragm of, 10.  
 — stage of, 9.

Microscope, arrangement of, for drawing, 26.

- binocular, 13.
- choice of a, 11.
- clinical, pocket or class, 14.
- dissecting, 14, 17.
- essential points in construction of, 11.
- makers of, 263.
- simple and compound, 5.
- student's, 10.
- how to examine objects in, 73.
- apparatus necessary in a, 12.
- travelling, 13.

Microscope objects, preparers of, 263.  
Microscopical investigation, chemical analysis in, 123.

Mirror, 8.

Molecules, 246.

Moulded glass cells, 71.

Mollusca, injecting, 117.

Mounting objects in Canada balsam, 98.

- apparatus for, 258.
  - in the dry way, 91.
  - in fluid, 92.
  - preparations in large glass cells, 56.
  - sections of bone, 84.
- Muscular fibre, 77.

NACHET, microscope maker, 263.

Naming preparations, 105.

Naphtha and creosote solution, 60.

Natural injections, 108.

Needles, 50.

— for passing thread round vessels, 109.

Negative eye-piece, 6.

Nerve fibres, 94.

Neutral tint-glass reflector, 27.

New views relating to structure and growth, 212.

Newt, kidney of, 79.

Nicot's prism, 24.

Nitrate of barytes, 135.

— of silver, 135.

— for staining tissues, 193.

Nitric acid, 130.

— effects of, upon animal structures, 132.

NOBBER's micrometer lines, 86.

Notes of observations, 246.

OBERHAUSER, microscope maker, 263.

Object-glasses, 6.

— on ascertaining the magnifying power, of 38.

Objects, drawing of, magnified with very high powers, 221.

— importance of examining, various ways, 86.

— illumination of, magnified by very high powers, 220.

— examined in different media, 24.

— simple method of measuring of, 37.

— to ascertain diameter of, 38.

Observers, delineating their own work, importance of, 32.

Oil-globules, 100.

Opaque injections, 109.

Operation of injecting, 117.

OSBORN, Lord S. G., his process of staining tissues, 190.

PAPER, tracing, 29.

Paper retracing, 29.

— transfer, 30.

Parabolic reflector, 22.

Pewter plate for grinding glass, 67.

Phosphates of lime, 139.

— triple or amoniac-magnesian, 129.

Photography, application of, to micro-

scopy, 149.

— history of, 150.

— apparatus, 153.

— Mr. Wenham, his arrangement, 151.

— Mr. Delves, his arrangement, 151.

— Mr. Shadbolt, his arrangement, 155.

— Dr. Maddox, his arrangement, 153.

— Drs. Abercrombie and Wilson, their arrangement, 159.

— of the illumination—sunlight, 161.

— of the illumination—artificial light, 162.

— of focusing, 163.

— the object-glasses, 164.

— stereoscopic photographs, 166.

— chemical solutions, 168.

— collodion, 168.

— nitrate bath, 169.

— developing solutions, 170.

— fixing solutions, 171.

— practical manipulations, 172.

— cleaning the glass-plates, 172.

— arranging the camera, 173.

— inserting the plate, 176.

— developing the image, 148.

— varnishing the plate, 180.

— cleaning old plates, 181.

— increasing intensity of negative, 181.

— of printing, 182.

— preparing the paper, 182.

— toning solution, 183, 185.

— fixing solution, 186.

— of mounting prints, 186.

Photographs of microscopic objects for magic lantern, 187.

PILLISCHER, microscope maker, 263.

Pins for dissecting, 51.

Pipe, injecting, 108.

Pipettes, 102.

Plate-glass slides, 51.

PLOEST, microscope maker, 263.

Polarized light, 4.

Polishing thin sections of hard tissues, 84.

Positive eye-piece, 6.

Potash, 133.

— and soda solutions of 203.

POWELL and LEALAND, microscope makers, 263.

Powers, use of very high, 215.

Practical operation of preparing hard tissues for examination with the highest powers, 209.

— soft tissues, do. do., 204.

Preparation, new methods of, 189.

— adapted for researches with the aid of the highest power, 195.

Preparations mounted in the dry way, 91.

— aqueous fluids, 92.

— Canada balsam, 98.

Preparers of microscopic objects, 263.

Preparing portions of injected preparations for microscopical examination, 121.



- Preparing embryonic tissues for examination with the highest powers, 212.  
 — hard tissues, do., do., 209.  
 — soft tissues, do., do., 204.  
 — objects for examination, 81.
- Preservation of objects in aqueous fluids, 58, 92.  
 — of crystals, 144.
- Preservative fluids, 58.  
 — GANNAL'S solution, 63.  
 — gelatine and glycerine, 62.  
 — glycerine, 58.  
 — GOADBY'S solution, 62.  
 — other saline solutions, 63.  
 — solution of chromic acid, 61.  
 — solution of naphtha and creosote, 60.  
 — spirit and water, 58.  
 — THWAITES' fluid, 59.
- PRICE'S patent glycerine, 59.
- Printers, 263.
- Prussian blue, 113.  
 — advantages of employing, 113.  
 — composition of, 114.
- QUEKETT, Mr., his arrangement for condenser, 23.
- RAZOR, 50.
- Reagents, 129.  
 — alcohol, 129.  
 — ether chloroform, 129.  
 — effects of, 130.  
 — acetic acid, 130.  
 — hydrochloric acid, 130.  
 — nitric acid, 130.  
 — sulphuric acid, 130.  
 — sulphuric acid, effects of on organic structures, 131.  
 — potash, 133.  
 — soda, 133.  
 — soda, effects of, on organic structures, 133.  
 — ammonia, 134.  
 — nitrate of barytes, 135.  
 — nitrate of silver, 135.  
 — oxalate of ammonia, 135.  
 — iodine solutions, 135.  
 — application of, to minute quantities of matter, 137.  
 — effects of, upon animal structures, 131.  
 — kept in small glass bulbs with capillary orifices, 137.
- Red injections, 110.
- Reflected light, 20.
- Reflector, parabolic, 20.
- Retort stand, 48.
- Retracing paper, 29.
- RICHARDSON, Dr., method of preserving tissues in nitrogen, 63.
- Rings, brass, for cutting circular pieces of thin glass, 67.
- ROSS, microscope maker, 263.
- Rotifers, examination of, 103.
- Round cells, 72.
- SALINE preservative solutions, 63.
- SALMON, Mr., microscope maker, 263.
- Salts, various metallic, for staining tissues, 194.
- Scalpels, 49.  
 — double-edged, for cutting thin sections, 49.  
 — SCHIEK, microscope maker, 263.  
 — SCHMIDT'S goniometer, 44.  
 — Scissors, 50.  
 — for cutting thin sections, 50.  
 — Sealing-wax varnish, 53.  
 — Sections of hair, longitudinal and transverse, 84.  
 — of bone, 84.  
 — of wood, 85.  
 — of shell, 85.  
 — of teeth, 84.  
 — of hard tissues, 83, 84, 85.  
 — of soft tissues, cutting, 82.
- SHADBOLT, Mr., apparatus for making cells, 66.
- Shades, 52.
- Shell-lac, solution of, 53.
- Siliceous skeletons of diatomaceae, 101.
- Silver, nitrate of, 135.
- Simple method of measuring objects, 37.
- Size and gelatine for injecting, 109.  
 — gold, 53.
- Slides, 51.
- SMITH and BECK, microscope makers, 263.  
 — lamp, 25.
- Soda, caustic, 145.  
 — and alcohol, for hardening tissues and making them transparent, 146.  
 — and potash, solution of, 203.
- Soft tissues, cutting thin sections of, 82.  
 — Practical operation of preparing for examining with the highest power, 204.
- Solution of chromic acid, and bichromate of potash, 203.  
 — of potash and soda, 203.  
 — colouring with glycerine, 202.
- Spherical aberration, 7.
- Spot-glass, 22.
- Spinal cord, method of obtaining sections of, 146.
- Spirit-lamp, 47.
- Spirits of wine, methylated, 58.
- Stage micrometer, 36.  
 — of microscope, 9.
- Standards of measurement, 39.  
 — foreign conversion of, 39.
- Staining tissues, 190.  
 — process followed by the Rev. Lord S. G. OSBORNE, 190.  
 — THIRSCH, his method, 191.
- Starch globules, 102.
- Steam bath, 48.
- Steel mirror, 8.  
 — disk, 27.
- Stone, drawing on, 30.  
 — engraving on, 30.  
 — for grinding thin sections of hard tissues, 84.  
 — lithographic, 31.
- Stopcocks, 109.
- Stops, 21.
- Structure of tissues, general observations on, 76.  
 — new views relating to, 212.
- Syringe for mercurial injections, 116.  
 — injecting, 108.
- Syrup, action of, on tissues, 200.  
 — and glycerine, 200.



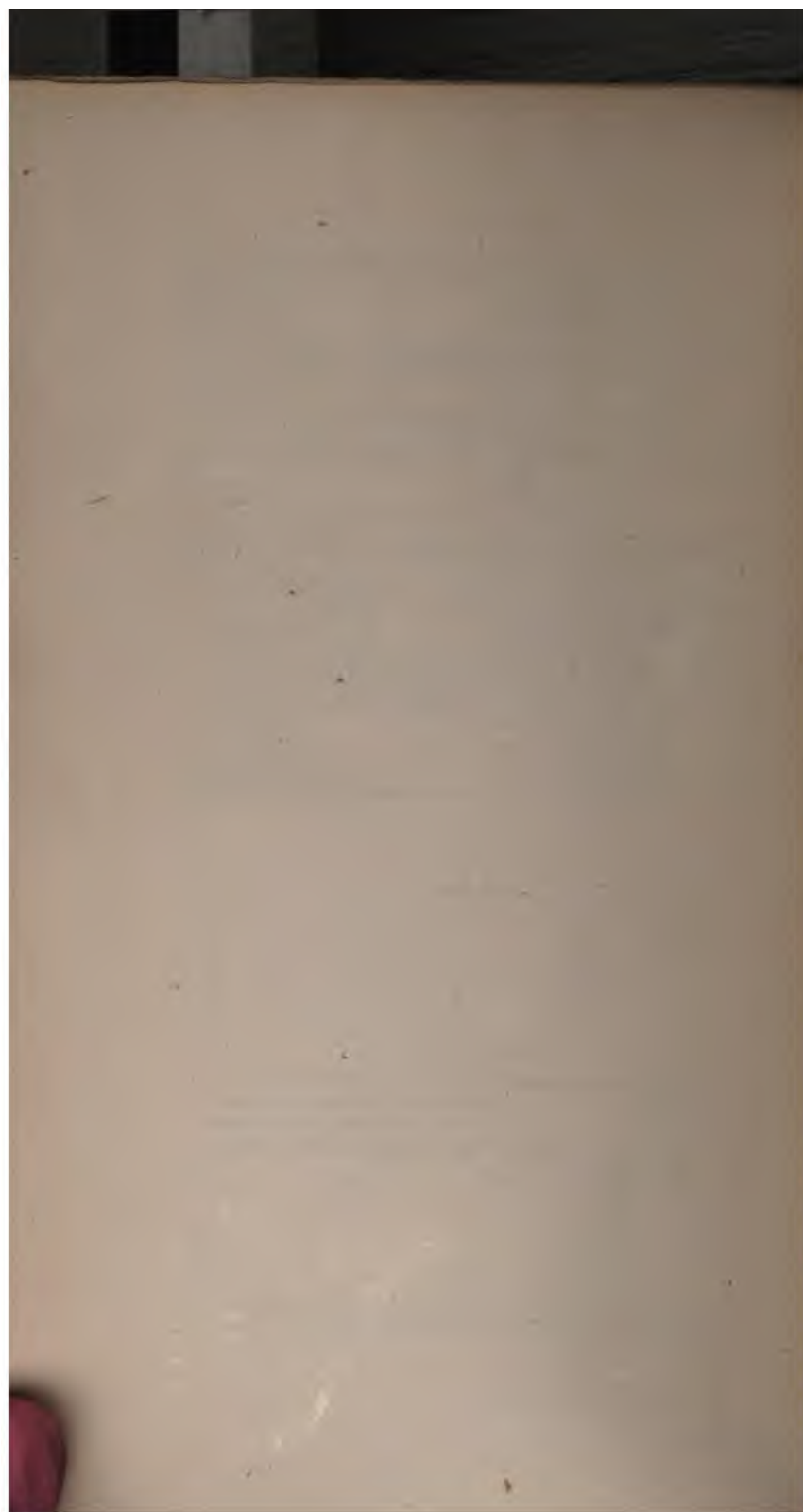
- TABLES for practising manipulation, 104.  
 Tablets upon which dissections may be pinned out, 82.  
 Tannin, 192.  
 Tea leaves, 245.  
 Teeth, sections of, 84.  
 Testing for chlorides, phosphates, sulphates, carbonates, lime, magnesia, 139.  
 Test objects, 36.  
 — tubes, 106.  
 Tests kept in bottles, or in small bulbs, 137.  
 Tests, method of applying, to microscopical examination, 138.  
 THIERSCHE, his method of staining tissues, 191.  
 Thin glass, 51.  
 — cells, 68.  
 — cover, placing it upon the cell, 95.  
 THWAITES' fluid, 59.  
 Time after death when tissues should be examined, 80.  
 Tissues, demonstration of, 78.  
 — embryonic, practical operation of preparing of, for examination with the highest powers, 212.  
 — hard, do., do., do., 209.  
 — soft, do., do., do., 204.  
 — which may be mounted in air, 91.  
 — which may be mounted in fluid, 92.  
 — which may be mounted in Canada balsam, 98.  
 Tracing paper, 29.  
 Transfer paper, 30.  
 Transmitted light, 23.  
 Transparency of objects, 217.  
 Transparent objects, illumination of, 23.  
 Travelling microscopes, 14.  
 Triple phosphate, 139.  
 Tripods, 48.  
 Triton, kidney of, 79.  
 Troughs for examining zoophytes, 72.  
 VALENTIN'S knife, new form of, 50.  
 Vallisneria, examination of, 96.  
 Varnish, sealing-wax, 53.  
 Vermilion, 110.  
 Vessels, examination of, 107.  
 VIII, 94.  
 Viscid media, for dissecting tissues in, for examination with the highest powers, 204.  
 Vivaria, 97.  
 Vorticella, 103.  
 WARINGTON, Mr., his microscope, 13.  
 Wash-bottle, 104.  
 Watch-glasses, 52.  
 Water-bath, 48.  
 Wax and gutta percha, tablets of, 82.  
 WHITE, Mr., his arrangement for fixing on the thin glass cover, 95.  
 White injections, 100.  
 — light for illumination, 25.  
 Wood, sections of, 85.  
 — blocks for engravings, 29.  
 — engravers, 263.  
 Wooden forceps, 51.  
 YELLOW injections, 110.  
 ZOOPHYTES, examination of, 72, 103.

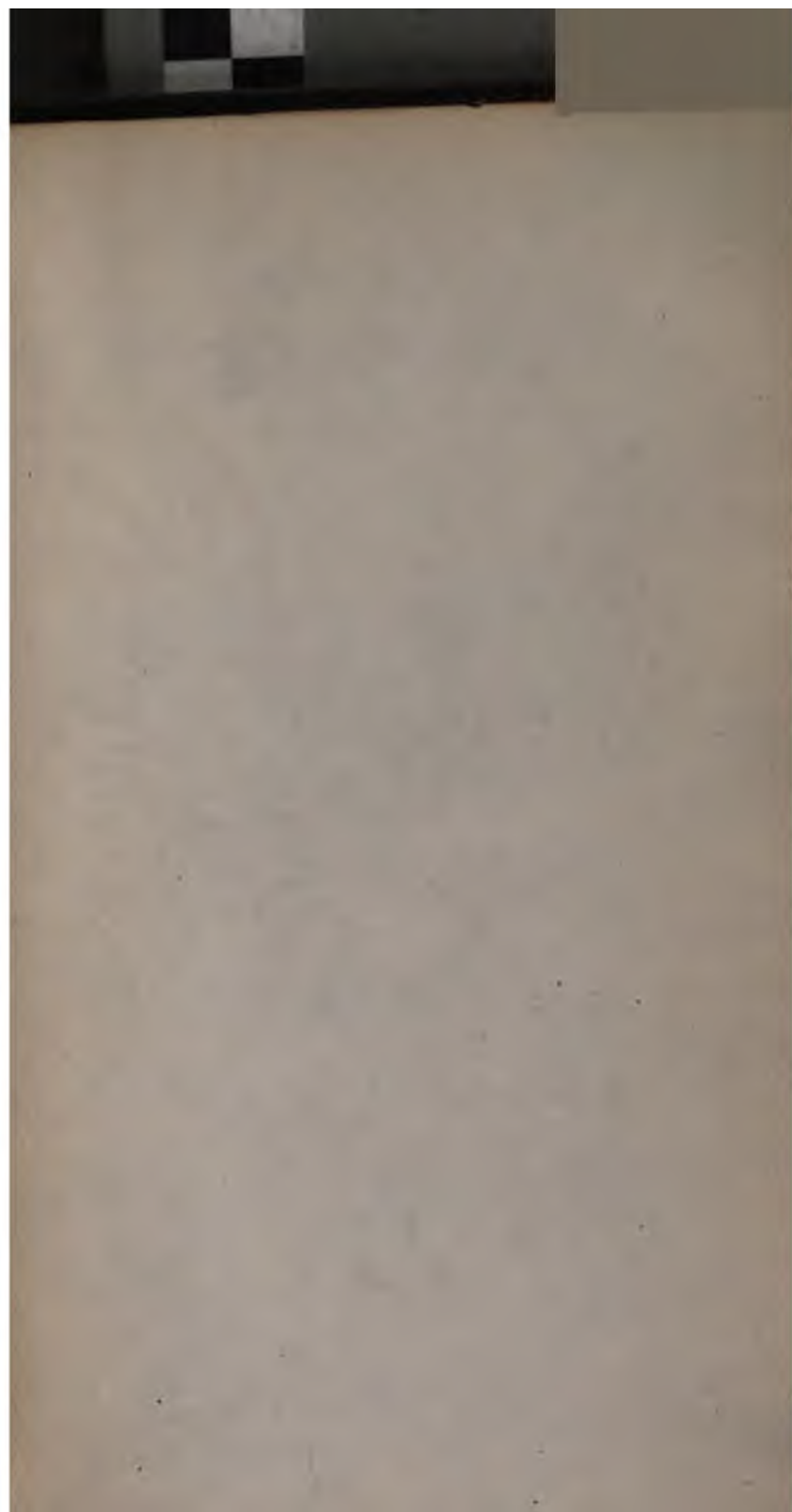
THE END.

#### ERRATA.

- In explanation at foot of frontispiece, for '*licmophora*' read '*licmophora*.'  
 Page 10, § 15, line 5, for '*first*' read '*new*.'  
 Page 28, line 21, insert '§ 62, p. 37.'  
 In Plate XXXIV, for '*speculum*' read '*operculum*.'









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